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# Eco-design of Spirulina solar cultivation: key aspects to reduce environmental impacts using Life Cycle Assessment

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# **Abstract**

To identify action levers that improve the environmental balance of producing Spirulina biomass, this work presents an LCA study of a modelled Spirulina cultivation coupled with Morris sensitivity analysis on the model parameters. The study concerns a hypothetical cultivation surface of 388.5 m<sup>2</sup> in open and covered raceway ponds (ORP and CRP) under greenhouse located in the city of Nantes in France. Several environmental indicators were evaluated, though GWP (global warming potential) was chosen for deep results discussion. Results showed a large variability of GWP (global warming potential) indicator for both culture modes (from 15.5 up to 67.3 kg eq. CO<sub>2</sub>/kg biomass dw) depending on the three most impacting parameters (the use of a thermal regulation system for the culture medium, the low setpoint temperature regulation and the annual production period). Moreover, for both ORP and CRP systems, GWP reduction per kg of biomass is reached by avoiding all impacts related to thermal regulation (i.e. thermal energy, heat pump, etc.) and simultaneously yielding high biomass productivity. Hence, annual cultivation should be extended as long as suitable solar conditions are available for the growth of microalgae (high and long radiation and high ambient temperature). Finally, both ORP and CRP systems reached similar annual production but showed different GWP impact since (i) their needs of thermal energy are opposite in warm and cold periods and (ii) the impact related to the CRP infrastructure is almost twice compared to the OPR infrastructure due to CRP the reactor cover.

*Keywords*: Spirulina, LCA (Life Cycle Assessment), microalgae solar cultivation system, microalgae growth modelling.

# **Abbreviations**

Acronym	Signification
CRP	Covered Raceway Ponds
dw	Dry weight
IF	Intermediate Flows
FU	Functional Unit
GWP	Global Warming Potential
LCA	Life Cycle Assessment
ORP	Open Raceway Ponds
PBR	Photobioreactor
SCPM	Summer-Centred Production Months
Tcmlow	Low setpoint temperature of culture media

## I. Introduction

Arthrospira platensis (usually named "Spirulina") is a blue-green cyanobacteria that can be naturally found in different aqueous media such as fresh and seawater, brackish water, marshes, sable and soil (Ciferri, 1983). The natural proliferation of this photosynthetic microorganism in warm lakes of Mexico and Tchad as well as its harvest and massive consumption as food by earlier populations has been reported since the  $15^{th}$  century (Léonard 1966). Spirulina is nowadays widely commercially produced. At the beginning of this decade, an optimistic estimation of the global annual production neared 10,000 tons (Belay, 2013). Some studies have related the ingestion of this microorganism to nutritional and therapeutic benefits such as hepatoprotective (Ferreira-Hermosillo *et al.*, 2010) and anti-inflammatory effects (Deng and Chow, 2010). In fact, Spirulina has a high protein content (up to 70% dw, Campanella *et al.*, 1999) and is also naturally rich in vitamins (B12, E), provitamins ( $\beta$ -carotene), minerals (iron, magnesium) and antioxidants such as phycocyanine (Cuellar-Bermudez *et al.*, 2014).

Besides, the composition of Spirulina includes other substances such as lipids and polysaccharides that have an interest in other domains, ranging from green chemistry (*i.e.* biomaterials, bio fertilizers) to energy. Some studies have in fact observed the aptitude of the Spirulina biomass to be used as a feedstock for biogas (Aramrueang *et al.*, 2016), biodiesel (Sumprasit *et al.*, 2017) and hydrogen production (Ainas *et al.*, 2017). Besides, some authors have pointed out encouraging results related to the biosorption capacity of Spirulina to remediate water contaminated with synthetic dye at batch

laboratory scale (Deniz and Kepekci, 2015). Likewise, Spirulina has shown certain ability to treat highly polluted wastewater from anaerobic digestion (Sankaran and Premalatha, 2018).

Much of the interest raised worldwide in the last few decades by microalgae biomass (here prokaryote cyanobacteria and eukaryote microalgae), is partially due to their high photosynthetic efficiency compared to conventional vegetal crops (between 10 to 20% versus e.g. less than 0.5% for switchgrass which is considered the fastest growing terrestrial plant, Li *et al.* 2008). Furthermore, to supply their metabolic needs for inorganic carbon, these microorganisms are able to uptake dissolved CO<sub>2</sub> which is an additional feature to counteract climate change.

However, within a global context of environmental deterioration (*i.e.* greenhouse effect, ecosystems pollution, exhaustion of limited resources), and the quest for the development of a sustainable industry, the operations related to growing microalgae processes need to demonstrate high yields as well as viable environmental performances compared to those attained by the manufacture of conventional or equivalent products.

A few studies have used the Life Cycle Assessment methodology (LCA, ISO 14040, 14044, 2006) to evaluate the environmental impacts of processes related to Spirulina and to identify the processes "hotspots", from the biomass growth to the transformation into different alternative products: edible tablets (Ye et al., 2018), phycocyanin (Papadaki et al., 2017), food and feed (Smetana et al., 2017), and biogas (Rodríguez et al., 2018). However, these previous studies (summarized in Table 1) are limited to some fixed operating conditions and do not investigate the variability of the operational parameters. Indeed, when processes include several correlated parameters that have certain variability, evaluating only a few scenarios appears to underrepresent the system heterogeneity and consequently leads to incomplete results and analysis, hence restricting and probably pre-orienting the choices of the decisions makers to reduce the environmental impacts. In this line, the main goal of this study is to couple a model of Spirulina biomass growth to LCA methodology in order to identify key operational aspects allowing the improvement of the environmental balance. These models should include parameters representing specific operational conditions to help make appropriate decisions in a given culture context.

This sort of approach has been already developed to link detailed operational models with LCA, and has been applied on a case study related to hemp crop production (Andrianandraina *et al.*, 2015) and its manufacture as an insulating material for buildings (Senga-Kiessé *et al.*, 2017). More recently, it has also been implemented to identify key parameters for the design of cement concrete (Ventura *et al.*, 2020).

Table 1. Synthesis of LCA literature results related to the cultivation of Spirulina

Author	Location of the production and culture mode	Studied species, culture conditions and considered stages	FU and LCA methodology	Inventory data	Results (GWP)*	Remarks
Smetana et al., 2017	Berlin, Germany.  ORP of 300 m³, annual production: 1.4 tons	C. Vulgaris and A. Platensis; Cultivation, flocculation, centrifugation.	1 kg of microalgae biomass (10-15% dw). ReciPe method using SimaPro software and Ecoinvent database 3.1	Modelling of pilot industrial scale based on relevant literature data and experimental data (lab and pilot scale).  Heating and cooling for cultivation steps included.	Chlorella in ORP: 28.08 kg CO <sub>2</sub> eq. per kg of biomass (20% dw)  Spirulina in ORP: 22.5 kg CO <sub>2</sub> eq. per kg of microalgae (20% dw)	Reduction of heating in ORP in more favourable climate conditions could reduce GWP (c.a 20%).
Ye et al., 2018	Beihai, south of China.  ORP, 20 tons/month, sunshine hours per year: 2009	A. Platensis; solar conditions.  Inoculation, cultivation, harvest, dewatering (filtration, spray drying), tablet manufacturing.	1 kg of Spirulina tablets (95% dw). TRACI 2 method and Ecoinvent database.	Based on algal tablets from real production plant. Biofuels for heating not considered (since they come from farm wastes). Wastewater not counted since discharged into fishpond. Construction materials of PBRs are not accounted.	1.6 kg eq. CO <sub>2</sub> per kg of microalgae (20% dw). Only 15% of this impact is not related to cultivation stage	Nutrients (urea, sodium bicarbonate, sodium nitrate, etc.) are responsible for 80% of GWP (as for most indicators).
Rodríguez et al., 2018	Unspecified location. Batch cultivation (50 mL) and continuous (10L) experiments. Light provided with LED lamps. Scaled-up system: ORP of 1129 m³. Light source unspecified.	A. Maxima, T <sub>culture</sub> =20°C, pH=9.4. Cultivation and dewatering (centrifugation). 5%wt of solids concentration after dewatering.	1 kg of dry biomass produced in a plant with capacity of 1 kg/h. CML method using Gabi software and Ecoinvent database 2.2	Based on experimental data, culture growth was modelled using Monod kinetic model. Scaled-up using super Pro Designer 9.5	$6.8~kg$ eq. $CO_2/kg$ biomass $20\%$ dw). Nutrient production shows the largest contribution to GWP $(6.4~kg~CO_2$ eq./kg biomass $20\%$ dw)	Substitution of mineral fertilizers as nutrient source is the key to improve the environmental performance

ORP: Open Raceway Pond

**GWP: Global Warming Potential** 

<sup>\*</sup>Results from other studies are recalculated in this table in order to present them here in respect to 20% dw, as in the present study.

In this article, we aim to apply a similar approach to the cultivation of Spirulina by combining a model of biomass surface productivity in solar conditions (Pruvost and Cornet, 2012) and a thermal model that estimates temperature variations of the culture (Goetz et al., 2011), with LCA and sensitivity analysis. The coupled model is used to identify operational key parameters for the reduction of the process environmental impacts and then to compare various culture scenarios. Two configurations under greenhouse are studied and compared: Open and Covered Raceway Ponds (ORP and CRP). While the former is a widely implemented and assessed technology for the microalgae culture (Collet et al., 2015), the latest is an alternative raceway pond with a transparent cover that could allow to save heat and water and whose more recent utilization has not been yet environmentally evaluated (according to our knowledge). This article focuses on the Global Warming Potential (GWP) indicator, though additional results concerning other impact categories are presented in Supplementary Data. In addition to minimizing GWP, the microalgae cultivator (i.e. the decision-maker) has other interests: increasing its surface productivity thus optimising the production per unit of cultivated surface and/or increasing its total annual production and thus increasing its sells. Thus, the study explores these three aspects: surface productivity, annual production and GWP.

#### II. Materials and Methods

# II.1 Description of the approach

The implemented methodology (based on Andrianandraina *et al.*, 2015) follows six main steps:

- 1. Developing a detailed model of the foreground system, which in contrast to the background system, is the part of the studied system under the direct influence of decision makers (Frischknecht, 1998); this step is detailed in section II.3;
- 2. Classifying the model parameters of the studied foreground system as controllable or not by the decision maker; the parameters classification is presented in Supplementary Data (Table S 14);
- 3. Identifying and assigning a probability distribution to all parameters; this step is detailed in the Supplementary Data (Table S 14);
- 4. Performing a Sensitivity Analysis, through the calculation of Morris indices, on the listed parameters of the model in order to qualify their influence on the different environmental indicators, this step is described in section II.4;
- 5. Identify action levers as controllable foreground parameters as key action possibilities for the decision maker in order to reduce environmental impacts as a result of sensitivity analysis detailed in section III.1;

6. Defining and comparing impact indicators of the most favourable scenarios for which controllable foreground parameters are set to suitable values; this is detailed in sections III.2 and III.3.

To conduct this approach, a computer simulation program was developed using Matlab®. The main modules of this program are resumed herein. The program simulates the microalgae production during a complete year in a given geographical location, using a parametric model of microalgae growth considering the variability of different culture parameters (e.g. length of the production period, thermal regulation of culture media, percentage of water separation after harvest). Subsequently, it calculates inventory flows (IFs) associated to the annual production of biomass (e.g. water, salts, electricity, thermal energy, cleaning products, infrastructures, etc.) according to other foreground operational parameters (e.g. fraction of water recycling, coefficient of performance of the heat pump used for heating). The IFs are then connected to the Ecoinvent 3.3 cut-off database (Weidema et al., 2013) to obtain inventories corresponding to the background system. Next, the environmental impacts of the system are calculated. Finally, a sensitivity analysis module is able to run simulations on the LCA model to identify action levers.

## **II.2** Life Cycle Assessment

#### II.2.1 Goal and Scope

Our foreground system is the cultivation process of Spirulina in two solar culture modes under greenhouse: Open and Covered Raceway Ponds (ORP and CRP). Our functional unit (FU) is the production of 1 kg of Spirulina biomass (dw: dry weight) contained in wet slurry (20% dw). For simplification purposes it will be henceforth noted as 1 kg of biomass (dw).

The LCA is performed on the 'Cradle to Gate' system illustrated in Figure 1. The foreground system is represented in the central frame of this figure. The solar culture modes are assessed on the basis of a surface culture of 388.5 m². All simulations of the microalgae production were performed by considering the annual meteorological conditions of the city of Nantes in France. A full and detailed description of foreground processes is provided in the Supplementary Data (§ I). Above and below the central frame are represented the background processes, which provide input flows (e.g. water, electricity, nutrients, etc.) and undertake output flows (e.g. wastewater treatment) of the foreground system.

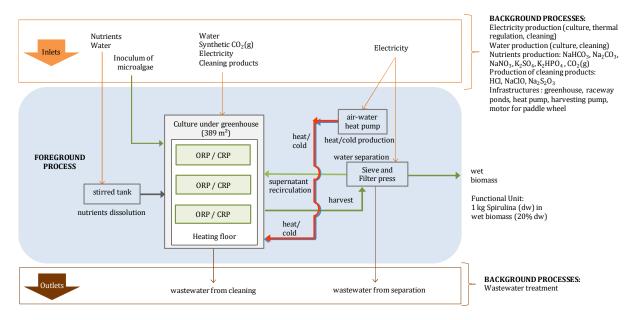


Figure 1. Representation of the Spirulina production process in this study

The inventory of the foreground system has been divided into 3 sub-inventories: Process, Cleaning and Infrastructure. Full details of inventory data and models are provided in the Supplementary Data (§III, Table S 4 to Table S12).

Our impact assessment method follows the International Reference Life Cycle Data System (ILCD) recommendations (European Commission, Joint Research Centre, 2012). Indicators are listed in Table S 13 in Supplementary Data.

The features of the cultivation facilities and the related operation data used are based on actual solar culture modes installed and operated in the city of Asserac (France), by the Algosource group.

#### II.2.2 Limitations

It should be noted that some IFs are not included in these inventories due to a lack of sufficiently robust data. As a result, their impact on the environment is not accounted for in the study. These flows are listed here below:

- For processes: Seeding inoculum of Spirulina, stirring energy for the preparation of the culture medium (salts + water).
- For infrastructures: sieve filter, filter press. Energy of the construction process.

#### II.3 Dynamic modelling of the foreground system

The inventory of the foreground system is based on the simulation of the annual production of microalgae biomass. For a given set of parameters (production period of

the year, geographical location, pond configuration) the annual production is estimated by following the next three steps, further described in the next section:

- i. Calculation of the hourly biomass maximum surface productivity considering the dynamics of solar radiation
- ii. Calculation of the culture's temperature based on the dynamic heat exchanges under the greenhouse.
- iii. Calculation of the "corrected" hourly surface productivity based on the effect of temperature on the microalgae growth

# II.3.1 Maximum surface productivity model

The model to estimate the maximum surface productivity has been previously developed by Pruvost and Cornet, 2012. It results from coupling photosynthetic biological kinetic parameters of the microalgae with the incident solar radiation on the reactor surface at a given instant. It estimates the average maximum surface productivity of the biomass ( $\langle S_x \rangle_{max}$ ) with an error of about 15% as follows:

 $f_d$ , unlit volume fraction of the reactor (-), is 0 in solar culture.

 $\rho_{\text{M}},$  maximum photosynthetic yield (conversion of light energy into physicochemical energy, (-))

 $\overline{\varphi}$ , average mass quantum yield of photosynthesis (Z-scheme)  $(g/\mu_{mol-h})$ 

 $\alpha$ , linear scattering modulus (-), characterizes the fraction of reflected light at the culture interface, specific for a given microorganism

K, half-saturation constant of photosynthesis specific to the microorganism ( $\mu_{mol-h}$ /m<sup>2</sup>.s)

 $\overline{q_0}$ , mean incident light energy density ( $\mu_{mol-h}$  /m<sup>2</sup>.s), also called PFD (Photon Flux Density)

 $\bar{x}_{\mathrm{d}}$ , mean diffuse fraction of  $\overline{q_0}$  (-)

 $\overline{cos\theta}$ , specification of the solar radiation on the PBR, with  $\theta$  the incident angle defined from the outward normal of the PBR and the sun's course (-)

Parameters related to the photosynthetic yield and kinetic growth ( $\rho_M$ ,  $\overline{\phi}$ ,  $\alpha$  and K) can be considered time independent for a given type of microalgae. The parameters  $\overline{x}_d$ , ( $\overline{q}_0$ ) and  $\cos\theta$ , are dependent on the dynamics of solar radiation. These are actually determined by the total incident solar radiation (Gt), horizontal diffuse solar radiation

(G<sub>H</sub>) and normal diffuse solar radiation (G<sub>N</sub>) which can be provided by meteorological databases such as Meteonorm® (Meteonorm, Meteotest, Switzerland). Thus, it is possible to calculate, for a given geographical location, the hourly maximum surface productivity throughout any other period of the year. For instance, Figure S 3 in Supplementary Data shows the incident luminous energy density ( $\overline{q_0}$ ) and the average maximum surface productivity ( $\langle S_x \rangle_{max}$ ) of Spirulina for an ORP culture under greenhouse during a year (from January to December) and located in the city of Nantes in France.

For a culture in a greenhouse, this model considers that the incident light energy density on the reactor surface  $(\overline{q_0})$  is attenuated when light goes through the transparent surfaces composing the greenhouse. This attenuation is determined by a luminous transmittance coefficient ( $T_L$ , 0 < $T_L$  <1) characteristic of the greenhouse material. If  $T_L$  is equal to 0.5, only half of the entire incident light energy density is transmitted through the material and is therefore available inside the greenhouse.

Note that *Eq.1* only estimates the maximum biomass productivity, and is then only related to available light, as discussed in Cornet and Dussap 2009. If culture conditions such as medium, pH or temperature are not in the optimal range of the culture species, biomass productivity will be then lower. The same will be obtained if biomass concentration is too low. Available light will be not fully absorbed in the culture broth leading to a decrease in productivity. For cyanobacteria like Spirulina, those optimal conditions (including light attenuation conditions as discussed in Pruvost *et al.*, 2012) are rather easy to obtain in practice. Only the control of temperature could be challenging, especially when energy input for thermal regulation needs to be minimized (see Pruvost *et al.*, 2019 for a complete discussion). The effect of culture temperature on productivity must be considered, which is detailed below.

#### *II.3.2 Model of thermal exchanges in the greenhouse*

Temperature is known to affect deeply microalgae growth (Pruvost *et al.*, 2016). The biomass surface productivity obtained by *Eq.1* must then be corrected accordingly. Thus, the biomass productivity model is combined with a thermal model that states differential equations resulting from unsteady state energy balances based on the thermal exchanges that take place between the various media composing the surroundings of the reactor (as presented by Goetz *et al.*, 2011 for a flat panel and by Pruvost *et al.*, 2019 for a semi-buried open raceway).

For the ORP in this work, these media are: the roof of the greenhouse, the air inside the greenhouse and the microalgae culture aqueous media. The temperature of the culture medium is assumed to be homogenous and it is considered that the heat transfer by conduction between the culture medium and the floor is non-significant. For the CRP, two more media are considered: the reactor cover and the air between this cover and

the microalgae culture aqueous media. For each considered medium, the terms included in the energy balance are the medium enthalpy variation, the solar radiation (absorbed, transmitted and reflected) and the heat exchange with the surroundings by radiation, convection and water evaporation/condensation. Thus, heat exchanges are characterised by several of the medium's properties (e.g. absorbance, transmittance, reflectance, heat capacity and emissivity).

The differential equations of the thermal model are solved using the Matlab® 'ode23' tool (Ordinary Differential Equation) as well as the hourly meteorological data provided by Meteonorm® for the studied geographical location. Solving these equations allows to calculate the temperature of the different media over time during one year.

#### II.3.3 Thermal regulation of the culture medium and related energy consumption

The thermal model provides the option of setting up a temperature range inside which the culture of microalgae will be maintained (Pruvost  $et\ al.$ , 2019). When the thermal regulation is activated, the model establishes that outside this interval, there is no change of the culture's temperature ( $dT_{culture}/dt=0$ ). Due to heat exchanges between the microalgae culture and its surroundings in the greenhouse, the thermal regulation affects other media's temperatures in the greenhouse.

In order to estimate the energy required to maintain the temperature of the culture medium within the chosen temperature range, a specific term of 'energy for thermal regulation' is then added to the unsteady state differential equation of the energy balance expressed for the culture medium. The addition of this energy term (either heating or cooling), implies that the temperature of the culture medium no longer changes over time ( $dT_{culture}/dt=0$ ) when its value is outside the defined temperature range. As discussed in Pruvost  $et\ al.$ , 2019, this assumes a thermal regulation unit of sufficient power to consider an instantaneous and ideal thermal response of the system.

#### II.3.4 Corrected surface productivity model: Effect of culture's temperature

As previously mentioned, since the production of microalgal biomass is not only determined by their kinetic parameters and the available solar radiation, but also by the temperature within the bioreactors, this model considers the effect of non-optimal temperature on the decrease of biomass surface productivity, as given by Eq.1 (i.e. maximal value). Thus, results from Eq.1 on biomass productivity were corrected by an empirical correlation (an Arrhenius type equation) establishing that below the optimal culture's temperature (35°C for Spirulina), the maximum biomass surface productivity undergoes a reduction as the culture's temperature decreases. Details on the effects of this correction are provided in Supplementary Data (Figure S 4).

## **II.4 Sensitivity Analysis: Morris Indices**

The sensitivity analysis method used in our approach, is the Morris method (Morris, 1991) providing three indices:  $\mu*$ ,  $\mu$  and  $\sigma$ . It consists in simulating several random modifications (one at a time) of the values of the model parameters according to their variation range. For each simulation an Elementary Effect (EE) is obtained by calculating the ratio between the variation of one output value obtained by the variation of one input parameter. For a set of a sufficient number of simulations for each parameter,  $\mu$  is the mean value of EEs,  $\mu*$  is the mean of absolute values of elementary effects |EEs|, and  $\sigma$  is the standard deviation of EEs. For a given varied input parameter of the model, the interpretation of Morris indices can be done as follows:

- the  $\mu*$  indice provides a ranking of parameters according to their influence on the environmental impacts indicator: higher  $\mu*$ , the higher the influence of the parameter
- if  $|\mu| \sim \mu^*$ , the effect of the studied parameter is an increasing effect
- if  $\mu \sim -\mu^*$ , the effect of the studied parameter is a decreasing effect
- if  $|\mu| \neq \mu^*$ , the effect of the studied parameter is non-monotonic
- if  $\sigma \ll \mu^*$ , the effect is linear like
- if  $\sigma$  is not negligible compared to  $\mu*$ , the effect is not linear or there are some interactions between the studied parameter and others

To generate the random sampling matrix in this study, the variation space of each parameter was discretized into 10 levels and the number of simulations computed for each parameter was set to 30.

In order to implement the Sensitivity Analysis, ranges of variation of all parameters of the model have been defined. They are presented in Supplementary Data (Table S 14) with their probability distributions and annotations on their meaning.

# III. Results and Discussion

# **III.1 Sensitivity Analysis**

#### III.1.1 Culture in Open Raceway Pond

Table 2 presents the values of the Morris indices ( $\mu^*$ ,  $\mu$  and  $\sigma$ ) obtained for the parameters of the culture model in ORP under greenhouse, for the GWP indicator. This table is completed by a graph presentation provided in Supplementary Data (Figure S 8). Parameters are ranked from most to less influential on the GWP indicator per FU.

The most influent parameter is found to be the one that determines whether there is a thermal regulation for the temperature of the culture or not. Activating the thermal

regulation leads to an increase of the GWP indicator ( $\hat{\mathbf{t}}$ ). This parameter also presents the highest degree of interaction or non-linearity ( $\sigma$ =4.1). Indeed, this parameter is a discrete coded parameter which means that its increment from 0 to 1 does not have any physical signification but allows the system to operate under different conditions (e.g. the supply of electrical energy to produce heat or cold through a heat pump in order to regulate the culture's temperature). This explains its high interaction degree. We can note that the high influence of this parameter on GWP is in accordance with Pruvost *et al.*, 2019 who concluded to the large energy consumption that could be obtained when adding a thermal regulation unit, even in the case of open systems. Although such conclusion is highly dependent of the strain temperature range and outdoor culture conditions, thermal regulation energy was found in most cases higher than the energy absorbed from solar radiation.

Table 2. Morris in	dices for the ORP	process regarding	the GWP indicator.

N°	model parameter (culture in ORP)	μ*	μ	σ	effect
1	thermal regulation	25.2	25.2	4.1	Û
3	number of summer-centred production months (SCPM) per year	17.9	14.8	3.3	①①
2	low temperature setpoint of the culture medium under thermal regulation (Tcm <sub>low</sub> )	11.0	11.0	2.4	Û
6	ratio of PBR surface to greenhouse surface	1.8	-1.8	0.2	Û
7	greenhouse height	1.0	1.0	0.1	Û
4	depth of the culture medium in the PBRs	1.0	8.0	0.2	ŪÛ
11	coefficient of performance of the heat pump	0.9	-0.9	0.3	Û
5	greenhouse material (PE, glass, PC)	0.6	0.0	0.2	ŪÛ
10	ratio of recycled water	0.5	-0.5	1.1E-02	Û
8	biomass concentration in the PBR before harvesting	0.2	-0.2	1.5E-02	Û
9	biomass concentration after harvesting and filtering	1.2E-03	1.2E-03	1.0E-04	Û

<sup>♣</sup> Parameter whose increase leads to a decrease of the indicator impact per FU.

The number of summer-centred production months (SCPM) per year is found as the second most influential parameter on the GWP indicator. It has a non-monotonic effect ( $\mathbb{Q}^{\bullet}$ ) because  $\mu^* \neq |\mu|$ . In other words, increasing the value of this parameter could lead to either a decrease or increase of the GWP indicator. Hence an optimal value should be determined.

The third most influent parameter is found to be the "low temperature setpoint of the culture medium" ( $Tcm_{low}$ ). It shows a monotonic increasing effect: an increase of this parameter rises the GWP indicator.

A similar increasing effect is observed for the parameters "greenhouse height" and "biomass concentration after harvesting and filtering" but their influence is found to be less important.

<sup>1</sup> Parameter whose increase leads to an increase of the indicator impact per kg of FU.

Other model parameters such as "depth of the culture media" and "greenhouse material" present non-monotonic behaviours with respect to the GWP indicator. However, their influence on GWP is found less significant, especially concerning the type of material used for the greenhouse. For a better interpretation of the meaning of this non-monotonic effect of this discrete coded parameter, several simulations were run to examine the impact of using these three types of greenhouse material. Results presented in Supplementary Data (Figure S 9) for operation under thermal regulation, show that the different results tend to overlap and confirm the small influence of this choice on the GWP indicator.

For all other parameters (*i.e.* ratio of PBR surface to greenhouse surface, coefficient of performance of the heat pump, ratio of recycled water, biomass concentration in the PBR before harvesting), they have a decreasing effect, their increment leading to reduce the GWP indicator  $(\mathbb{J})$ , though their influence is found to be minor.

# III.1.2 Culture in Covered Raceway Pond

Table 3 presents the results of the Morris indices obtained for the model parameters in the CRP configuration. The three most influent parameters regarding the GWP indicator are similar to those found for the ORP configuration. Sensitivity analysis results related to other environmental indicators are presented in Supplementary Data in Table S 19 and Table S 20.

N°	model parameter (culture in CRP)	μ*	μ	σ	effect
1	thermal regulation	19.1	19.1	2.4	Û
2	low temperature setpoint of the culture medium under thermal regulation (Tcm <sub>low</sub> )	8.4	8.4	2.1	Û
3	number of summer-centred production months (SCPM) per year	8.1	-0.4	2.0	ŪÛ
5	greenhouse material (PE, glass, PC)	1.6	0.5	0.3	IJÛ
4	depth of the culture medium in the PBRs	1.3	-0.2	0.3	IJÛ
6	ratio of PBR surface to greenhouse surface	8.0	-0.8	0.1	Û
11	coefficient of performance of the heat pump	0.7	-0.7	0.2	Û
7	greenhouse height	0.7	0.7	4.3E-02	Û
10	ratio of recycled water	0.5	-0.5	1.3E-02	Û
8	biomass concentration in the PBR before harvesting	0.2	-0.2	1.4E-02	Û
9	biomass concentration after harvesting and filtering	4.2E-04	4.2E-04	7.8E-05	Ω

Table 3. Morris indices for the CRP regarding the GWP indicator.

Parameter whose increase leads to a decrease of the indicator impact per FU.

<sup>1</sup> Parameter whose increase leads to an increase of the indicator impact per FU.

## III.2 Generating scenarios for most favorable production conditions

The sensitivity analysis provides the three most influent parameters on GWP: (i) the choice to use or not thermal regulation, (ii) the choice of the low temperature setpoint Tcm<sub>low</sub> under thermal regulation, and (iii) the number of SCPM per year.

Further simulations are conducted in order to evaluate with more detail their influence on the GWP indicator, surface productivity and annual production as well as to obtain possible optimal values for SCPM which is found to be non-monotonic.

For these simulations, the values of all other parameters were set to their most probable value according to their probability distribution presented in Table S 14 in Supplementary Data (except for the material of the greenhouse, which was defined by default as Polyethylene).

# III.2.1 Effect of influent parameters on surface productivity

Figure 2 presents the simulated average surface productivity with and without thermal regulation for both types of solar culture modes. First of all, these results show a high variability of the average surface productivity (between 2.9 and 9.0 g/m $^2$ /d) depending on the extent of the annual production period, the thermal regulation conditions and the reactor configuration.

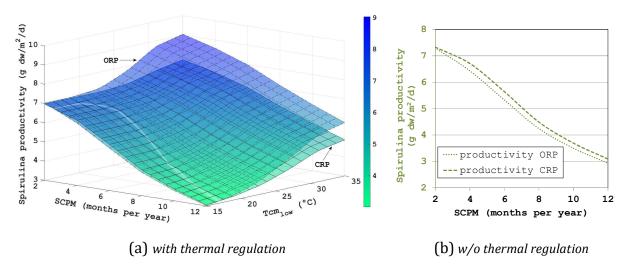


Figure 2. Simulation results for average surface productivity in ORP and CRP for different number of SCPM per year and low temperature setpoint of culture medium ( $15^{\circ}\text{C} \leq \text{Tcm}_{low} \leq 35^{\circ}\text{C}$ ) (a) with thermal regulation and (b) without thermal regulation. Simulations are run for 389 m² of surface culture under greenhouse located in the city of Nantes.

Under thermal regulation (Figure 2a), for a given annual production period (SCPM), the average surface productivity increases with the increment of the low temperature

setpoint of the culture ( $Tcm_{low}$ ), as defined by the productivity model described (cf. §II.3.4). Furthermore, for a given  $Tcm_{low}$ , higher surface productivities are reached for a production period of 2 months (SCPM=2), i.e. June and July. Indeed, for the city of Nantes, these are the 2 months accumulating the highest number of hours of sunshine (21% or 970h out of 4558h per year) and where the close-to-zenith position of the sun provides more favourable sunlight for microalgae growth (*i.e.* photon flux density ( $\overline{q_0}$ ) and incident radiation angle ( $cos\theta$ )). In that configuration, ORP maximum productivities are 7.0 and 9.0 g dw/m²/d, and CRP maximum productivities are 7.0 and 7.9 g dw/m²/d, for  $Tcm_{low}=15^{\circ}C$  and 35°C respectively. Lower productivities of CRP are due to the reactor cover: although transparent, it generates a condensed-water film that reduces the transmission of light to the culture media. Besides, when the production period is extended, the surface productivity decreases since the hours of sunshine per day decrease and the sun is more distant to the zenith position (lower values of  $\overline{q_0}$  and  $cos\theta$ ).

For instance, the months of January and December provide only 281 and 261 hours of sunshine respectively (together 12% of the 4558 annual sunshine hours) and during these hours the sun rises far from the zenith and provides a low incident radiation (low  $\overline{q_0}$  and  $\cos\theta$ ). Thus, an operation of 12 months per year (January  $\rightarrow$  December) would make the average surface productivity values the lowest because these low sunshine months are included in the production period.

Without thermal regulation (Figure 2b), the productivities obtained in ORP and CRP are very close. The surface productivity decreases progressively from 7.3 down to  $2.9~{\rm g}~{\rm dw/m^2/d}$  as the production period (SCPM) extends from 2 to 12 months per year. These values are equivalent to the ones obtained under thermal regulation with  ${\rm Tcm_{low}}=15^{\circ}{\rm C}$ .

#### III.2.2 Effect of influent parameters on biomass production

The simulation results concerning total annual biomass production with and without thermal regulation are presented in Figure 3.

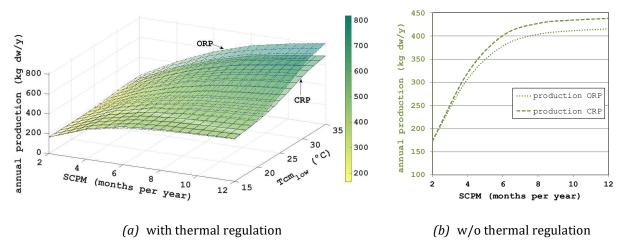


Figure 3. Simulation results for biomass annual production in ORP and CRP for different number of SCPM per year and low temperature setpoint of culture medium ( $15^{\circ}C \le Tcm_{low} \le 35^{\circ}C$ ) (a) with thermal regulation and (b) without thermal regulation. Simulations are run for 389 m² of surface culture under greenhouse located in the city of Nantes.

With thermal regulation (Figure 3a), the maximum annual production for ORP and CRP (respectively 819 and 691 kg/year) are obtained with a temperature of Tcmlow=35°C and logically with the longest operating period (SCPM=12, January→December). Besides, as expected, for a given number of SCPM, increasing the Tcmlow leads to an increase in annual production. For 12 SCPM, the annual production rises from 427 to 819 kg/year in ORP (+92%) and from 432 to 691 kg/year for CRP (+60%) when Tcmlow is incremented from 15 to 35°C.

Furthermore, the increment in annual production with the extension of the cultivation period is not linear, but tends towards a plateau. For instance, extending the production period from 2 months per year (SCPM=2, June→July) to 4 months per year (SCPM=4, May→August) in the ORP system with Tcm<sub>low</sub>=15°C, results in a production increment of 132 kg/year (from 166 to 298 kg/year), *i.e.* around 30% of the production achievable for 12 months of operation (427.1 kg/year) under the same Tcmlow. Moreover, if the period of operation is extended from 8 to 10 months or from 10 to 12 months per year, the annual production increases by approximately 15 kg or 11 kg respectively, which corresponds to nearly 3% at maximum production achievable for 12 months of operation at this temperature. These results are explained by low surface productivities obtained when the production period takes place away from the most favorable sunlight conditions. Quite similar values are found for the CRP configuration and Tcmlow=15°C. However, at higher temperatures, the CRP culture mode tends to provide lower annual productions than the ORP configuration which is caused by the reactor cover of the CRP that attenuates the light transmission to the microalgae culture. For an operation period of 12 SCPM and Tcm<sub>low</sub>=35°C, the CRP system provides 18% less biomass per year than the ORP configuration (691.4 and 818.5 kg/year respectively).

Without thermal regulation (Figure 3b), the annual production trend shows a strong increase from 2 to 8 SCPM, and then only a slight increment from 8 to 12 SCPM. For annual production periods between 6 and 12 SCPM, the annual production tends to be slightly higher for the culture in CRP compared to ORP. The small difference between these two culture modes can be explained by the capacity of the CRP system to maintain a higher culture temperature (annual mean culture temperatures are respectively 21.8 and 18.8°C) thanks to the reactor cover that avoids water evaporation and heat exchange by convection with the air inside the greenhouse. These trends are similar compared to those obtained under thermal regulation with Tcm<sub>low</sub>=15°C.

# III.2.3 Effect of influent parameters on Global Warming Potential

Simulation results of the GWP indicator per kg of biomass (dw) are presented in Figure 4. Under thermal regulation (Figure 4a), for a given production period, the GWP indicator increases, as expected from the sensitivity analysis, with the increase of the low temperature setpoint (Tcmlow). Indeed, the energy consumption is directly linked to this value.

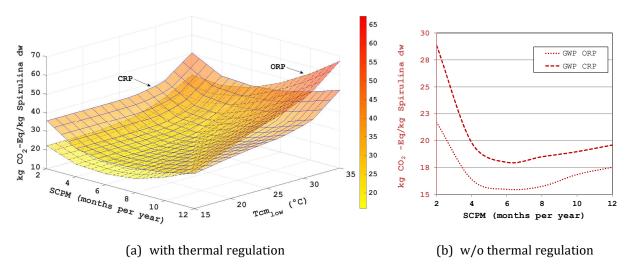


Figure 4. Simulation results for GWP indicator per kg of biomass (dw) in ORP and CRP for different number of SCPM per year and low temperature setpoint of culture medium ( $15^{\circ}C \le Tcm_{low} \le 35^{\circ}C$ ) (a) with thermal regulation and (b) without thermal regulation. Simulations are run for 389 m<sup>2</sup> of surface culture under greenhouse located in the city of Nantes.

Besides, at a given Tcm<sub>low</sub> (varied between 15 and 35°C), reducing the annual cultivation period (SCPM) shows a non-monotonic influence on GWP (in accordance with previous results, Table 2 and Table 3), with an optimal value of SCPM (around 6 months) for which GWP is minimum for both ORP and CRP culture modes.

For instance, under thermal regulation in ORP with  $Tcm_{low}=15^{\circ}C$ , shortening the annual production period (SCPM) from 12 months to 6 months, reduces the environmental impact by around 46% (from 29.3 to 15.8 kg  $CO_2$  eq. /kg biomass dw). However, further reduction of the annual operation period (SCPM) down to 2 months (June  $\rightarrow$  July) results in an increase in the GWP indicator up to 22.8 kg  $CO_2$  eq./kg of biomass dw (+ 41%).

To analyse the effect of the annual operation period (SCPM) on GWP impact, Figure 5 presents the simulation results for the heating and cooling energy required per year for thermally regulated cultures at Tcmlow=15°C, as well as the annual biomass production.

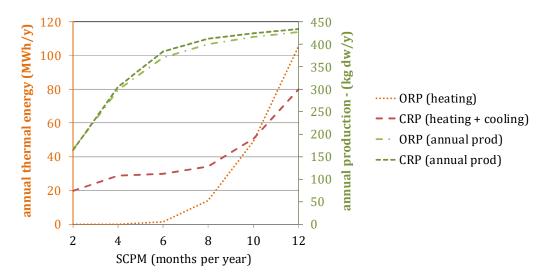


Figure 5. Simulated thermal energy required to maintain the culture at  $Tcm_{low} = 15^{\circ}C$  in ORP (heating) and CRP (heating+cooling) under greenhouse (389 m<sup>2</sup> of culture) and associated annual Spirulina production for different number of SCPM per year.

In Figure 5, for ORP with SCPM shorter than 6 months, heating energy remains close to zero, because ambient temperature is sufficiently high and solar radiation conditions provide sufficient heat to fulfill the thermal requirements to reach the setpoint temperature Tcm<sub>low</sub>. Simultaneously, the annual production rises significantly (+133%) when the annual operation extends from 2 to 6 SCPM per year (from 165 up to 385 kg dw/year respectively). In contrast, when SCPM exceeds 6 months, the annual production reaches a plateau (+13%), but energy demand to provide sufficient heat drastically increases (up to 106 MWh/year for 12 SCPM in OPR). Besides, though not shown in this graph, other resources and emissions are linked to this growing demand of thermal energy (heat pump, refrigerant R134a, leaks of refrigerant (Tetrafluoroethane emissions)), and contribute to the GWP impact.

For the CRP culture mode, the same observations can be made about the influence of SCPM. However, thermal energy requirements are different from the ORP culture mode, although their annual productions are found comparable. When the annual operation (SCPM) lasts between 2 and 8 months, the CRP culture requires cooling energy to respect Tcmlow. Instead, for longer annual operations that include the coldest months (SCPM>10) the CRP system requires less heating energy than ORP (80 and 106 MWh/year respectively) due to its cover that allows to keep the reactor's temperature without further energy demand. This explains why in Figure 4a the GWP impact related to the ORP system, tends to appear lower than GWP impact of the CRP for short annual operations (low SCPM) whereas for long annual production periods (high SCPM) it appears to be higher, this trend being more pronounced with the increment of Tcmlow, since the demand of thermal energy is increased.

Thus, on one hand, an increase of SCPM increases the biomass annual production. This will contribute to reduce GWP as it is calculated on the basis of the functional unit in this study: total GWP is divided by total produced biomass (dw). On the other hand, a longer SCPM period increases (i) the amounts of resources (intermediate flows) required for the culture operation (water, salts, electricity, paddle wheel, pumps, etc.) and, in the case of thermal regulation (ii) the energy requirements and related flows for maintaining a growing biomass at a given Tcmlow (thermal energy, heat pump, refrigerant, etc.). An equilibrium point, at which SCPM is around 6 months, is found when production almost reaches a plateau as shown in Figure 5: the annual production is then the highest in respect to the needed intermediate flows associated with the growth of this biomass.

Finally, without thermal regulation (Figure 4b), independently of the length of the annual operations (SCPM) the CRP system is found to have a higher GWP impact per kg of biomass than ORP (from +12% up to +33%) and that in spite of similar annual productions in both systems. This difference can be attributed to other features differentiating both cultivation systems, like their infrastructure itself, and whose GWP impact was not highlighted in the previous analysis, though it can significantly contribute to the total GWP as it will be shown in §III.3.2.

# III.2.4 Definition of most favourable scenarios

In the model, an increase of surface productivity can be reached by two means: the regulation of the culture's temperature and the increase of daily solar radiation intensity and availability (number of hours). However, these two means do not have the same environmental loads: whereas thermal regulation implies the need of additional energy, for a given geographical location the solar resource is only conditioned by the annual cultivation period. Thus, logically, the more favourable the solar conditions, the less needed the thermal regulation. This consequently leads to lower GWP values. This result can be generalized for all microalgae cultures, only the value of GWP would change for other species, especially if they require a different culture temperature than Spirulina.

Such analysis is then in accordance and complementary to others studies in the field (see Pruvost et al., 2019 for a similar discussion on the challenge to optimally produce microalgae outdoor all year round).

The detailed analysis shows that optimal value of SCPM is close to 6 months in order to obtain lower GWP. It also shows that the increase in production is small above these values, because surface productivity decreases due to shorter daily sunlight time, and lower sun positioning. Results also show that the use of thermal regulation is very influent on GWP increase. However, it appears unnecessary to be used during the summer (low SCPM), as both ORP and CRP have the similar productions with or without thermal regulation (see Figure 3). Thermal regulation appears useful to extend SCPM above 6 months, in order to increase production, only if the low temperature setpoint is at least 20°C (see Figure 3).

Based on this analysis, several specific and plausible production scenarios, corresponding to lowest values of GWP according to Figure 2, are proposed for both ORP and CRP (Table 4). These scenarios consider the same cultivation context described previously (i.e. area of 388.5 m² under greenhouse located in the city of Nantes, France).

Table 4. Specific simulated scenarios for the production of Spirulina in ORP and CRP under greenhouse (389  $m^2$  of culture surface) located in the city of Nantes, France.

	<i>S</i> 1	<i>S2</i>	<i>S3</i>	<i>S4</i>	<i>S5</i>
SCPM (months)	8	6	6	8	6
production period	March <del>→</del> Oct	Apr→Sept	Apr→Sept	March→Oct	Apr→Sept
thermal regulation	Yes	Yes	Yes	No	No
Tcm <sub>low</sub> (°C)	20	30	20	-	-

For each scenario, the estimated average surface productivity, annual production and related annual inventories of the intermediate flows (IFs), are presented in the Supplementary Data (Table S 22 and Table S 24 for ORP and CRP respectively). Likewise, the simulated environmental indicators (ILCD Midpoint method), associated to these inventories are presented in Supplementary Data in Table S 23 and Table S 25 (for ORP and CRP respectively). Results presented here next are related to the GWP indicator.

#### III.3 Evaluation of defined scenarios

#### III.3.1 Scenarios comparison

Concerning both ORP and CRP, the production scenario S2 would reach the highest impacts per kg of produced biomass on all indicators, except on the Land Use indicator (cf. Table S 23 and Table S25 in Supplementary Data). The GWP impact is 28.2 and 26.4 CO<sub>2</sub> eq./kg of biomass (dw) in ORP and CRP respectively. Scenario 2 is characterized by an important annual consumption of electrical energy (45.7 and 24.7 MWh/year for ORP and CRP respectively) exclusively related to temperature regulation (160.1 MWh/year

for heat in ORP and 43 MWh/y and -43.5 MWh/y for heat and cold respectively in CRP) to ensure a minimum culture temperature (Tcm<sub>low</sub>) of 30°C during 6 SCPM per year (April→September). In the same time, the operating conditions of Scenario 2 allow achieving the highest surface productivity for both ORP (7.4 g/m²/y and 527 kg Spirulina dw/y respectively) and CRP (6.3 g/m²/y and 450.9 kg/y respectively, cf. Table S 22 and Table S 24 in Supplementary Data). These estimated productivity values are comparable to experimental data obtained on site (Algosource's production site in Asserac).

For both ORP and CRP, the lowest impact values per kg of produced biomass (dw), except for the Land Use indicator (Table S 23 and Table S 25 in Supplementary Data), are obtained for the scenario 5 with similar results for both types of culture modes. GWP indicators for scenario 5 were 15.5 and 18 kg CO<sub>2</sub> eq./kg biomass dw respectively for ORP and CRP. This scenario operates for 8 SCPM and without thermal regulation. Although it provides lower annual productions than scenario 2 (378.8 and 401.4 kg/year for ORP and CRP), it also avoids all impacts related to the use of temperature regulation (i.e. thermal energy, use of heat pump and R134a and Tetrafluoroethane emissions due to leaks of R134a).

In order to identify the scenario that deals the best with this balance between high annual productions and low GWP impact per kg of biomass, the next steps were followed. First, the scenario providing both the highest annual production and the highest GWP indicator (Scenario 2 and ORP culture mode) was fixed as a reference. Variations of both production and GWP were calculated for the other scenarios with respect to scenario 2 (Table 5). The ratio of production loss to GWP decrease was then calculated to identify the scenario providing an adequate compromise between annual production and GWP impact per kg of biomass.

The most interesting ratio (lowest value=0.53) is obtained for scenario 4 (SCPM=8 months, no thermal regulation) with an ORP culture mode. Compared to scenario 2, production loss is -23%, and GWP decrease is -44%. The CRP culture mode of scenario 4 has a slightly higher ratio (0.55), with a production loss of -19%, but a GWP decrease of -34%.

Table 5. Spirulina annual production and GWP per FU for 5 different cultivation scenarios in ORP and CRP under greenhouse (389  $m^2$  of culture surface) located in the city of Nantes, France.

	Culture mode	<b>S1</b>	<b>S2</b>	<b>S</b> 3	<b>S4</b>	<b>S</b> 5
annual production	ORP	430.5	526.9	384.6	404	378.8
(kg biomass dw)	CRP	424.6	450.9	387	427.6	401.4
GWP	ORP	21.4	28.2	17	15.8	15.5
(kg CO2 eq/kg biomass dw)	CRP	23.8	26.4	22.4	18.5	18

nnaduation loga / 52	ORP	-18%	Ref	-27%	-23%	-28%
production loss / S2 <sub>ORP</sub>	CRP	-19%	-14%	-27%	-19%	-24%
CIMD doggood / C2	ORP	-24%	Ref	-40%	-44%	-45%
GWP decrease / S2 <sub>ORP</sub>	CRP	-16%	6%	-21%	-34%	-36%
ratio $\frac{production\ loss/S2}{quarter}$	ORP	0.76	Ref	0.68	0.53	0.62
GWP decrease/S2	CRP	1.24	2.26	1.29	0.55	0.66

#### III.3.2 Contribution of Intermediate flows to GWP indicator

Figure 6 and Figure 7 show the contribution fraction of the inventory flows to the GWP indicator obtained for the ORP and CRP respectively and the different evaluated scenarios.

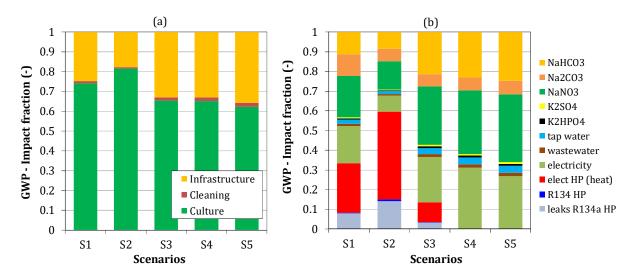


Figure 6. Contributions fractions of the IFs to the GWP indicator for the five simulated scenarios in **ORP** (389 m²) under greenhouse in Nantes, France. (a) IFs related to Infrastructure, Cleaning and Culture; b) IFs related to the Spirulina culture.

For the ORP system, the group of operations related to the biomass **Culture** (cultivation and harvest) is the main contributor to the GWP indicator (between 62 and 81%, Figure 6a). For scenarios operated under thermal regulation (S1, S2 and S3), the electricity related to the heating energy supplied by the heat pump, plays a major role in this impact (Figure 6b). Its contribution can reach up to 44% (S2). Associated to thermal regulation, the leaks of refrigerant R134a from the heat pump (Tetrafluoroethane emissions) also participate to this GWP impact (up to 14% of the total impact). Moreover, the contribution of electricity for culture mixing, biomass harvesting and filtering, vary from 8 up to 31%. Some of the salts added to provide the elementary nutriments participate importantly to this impact especially NaNO<sub>3</sub> (between 14% and 34%), followed by NaHCO<sub>3</sub> (8 and 25%) and Na<sub>2</sub>CO<sub>3</sub> (6 and 11%). The contribution of tap water and wastewater flows to the GWP impact appears to be minor (less than 3%).

On the other hand, the GWP impact fraction related to the **Infrastructures** varies between approximately 18 and 36% (Figure 6a). Most of this impact comes from the infrastructure of the bioreactor itself (between 55 and 69%), followed by the greenhouse infrastructure (between 24 and 30%) and by the heat pump infrastructure (from 2 up to 20%) whose contribution takes place when the thermal regulation is activated. The impact fraction of other considered infrastructures such as the engines for the paddle wheel and the harvesting pump appears to be negligible. To observe how much the materials composing the ORP and the greenhouse infrastructures contribute to their total GWP impact, see Figure S 5 and Figure S 7 in Supplementary Data. Near 30% of the GWP impact related to the ORP infrastructure comes from the concrete composing the screed of the heating underfloor followed equivalently (ca. 14%) by the PVC (structural), the EPDM (liner) and the waste management of the EPDM. Concerning the greenhouse infrastructure, approximately 75% of its GWP impact relates mainly to the structural steel, the remaining fraction (25%) being equivalently caused by Polyethylene and waste management of Polyethylene.

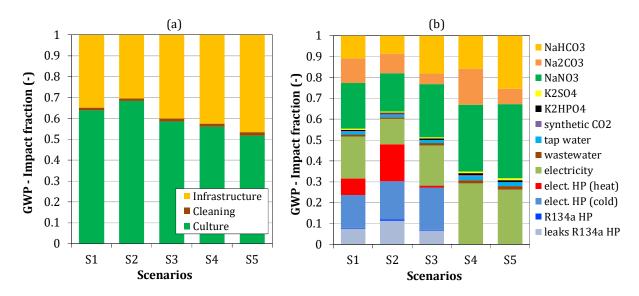


Figure 7. Contributions fractions of the IFs to the GWP indicator for the five simulated scenarios in CRP (389 m<sup>2</sup>) under greenhouse in Nantes, France. (a) IFs related to Infrastructure, Cleaning and Culture; b) IFs related to the Spirulina culture.

Regarding the CRP configuration, the GWP impact fraction related to the **Culture** operations varies from 51 up to 35% (Figure 7a), which is lower to the one observed for the ORP system. Contributions of the inventory flows to the total GWP impact are similar to the ones observed previously for the ORP system. Two additional flows are in this case part of the inventory: the electricity related to the cooling energy and the synthetic CO<sub>2</sub>. For scenarios where temperature regulation is activated, the electricity flow related to cooling energy is at least half of the total electricity related to thermal energy. It can reach up to 20% of the total GWP impact associated to the culture operations. The GWP

contribution of the group of flows related to the thermal regulation (heating, cooling, R134a, leaks of R134a) varies between 28 and 48%. On the contrary, the contribution of the synthetic  $CO_2$  on this impact is negligible (less than 1%).

Furthermore, the impact fraction associated to the CRP Infrastructures varies from 31 up to 47% of the total GWP impact (Figure 7a), which is higher than one obtained for the ORP configuration. This is partially due to the infrastructure of the CRP bioreactor that has almost twice the GWP impact found for the ORP configuration (cf. Table S 16 in Supplementary Data). In fact, the bioreactor infrastructure is responsible for at least 75% up to 81% of the total GWP impact related to Infrastructures. This significant impact caused by the CRP infrastructure helps to explain why in the absence of thermal regulation (b), the GWP impact related to the cultivation in the CRP system appears to be higher than the one estimated for the ORP configuration, independently of the annual operation period (SCPM). Besides, the remaining GWP impact related to the CRP infrastructure is caused by the greenhouse infrastructure (near 18%) followed by the GWP impact associated to the heat pump for cooling needs (about 3 to 8%) used when temperature regulation is activated. The contributions of the materials composing the infrastructure of the CRP bioreactor are shown on Figure S 6 in Supplementary Data. The Polycarbonate (PC) used for the reactor cover and whose service life is considered to be shorter compared with other infrastructure materials, is the main contributor to this GWP impact (41%), followed by HDPE used for the reactor structure, isolation tubes and paddle wheel (17%), concrete for the screed of the heating underfloor (16%) and waste management of HDPE (15%).

Besides, the GWP impact related to the **Cleaning** steps appears to be negligible (lower than 2% of the total GWP impact) for both ORP and CRP.

# **IV. Conclusion**

The purpose of this study was to identify key operational parameters of the Spirulina production in two solar bioreactors (ORP and CRP) under greenhouse in order to reduce their potential environmental footprint. To this aim, we have developed a simulation platform that integrates a validated model of microalgae surface productivity, coupled to a thermal model, with LCA methodology and sensitivity analysis. The implemented approach can be used as a decision tool to guide biomass production with low environmental impact. The main findings of this study, mainly focused on the GWP indicator, can be summarized as follows:

 Simulations of average surface productivity, annual production and GWP per unit of produced biomass showed a large variability depending mainly on three most influential parameters: the culture annual operation period (centred on summer

months), the use of thermal regulation, and the low temperature setpoint of culture medium ( $T_{cmlow}$ ) when thermal regulation is used.

- To optimize the GWP impact per unit of produced biomass (i.e. to minimize the use of resources and emissions per FU), the results showed that annual operations should favour periods where solar conditions provide sufficient heat and light. The use of the thermal regulation and the increment of T<sub>cmlow</sub> may help to reach higher annual productions, especially during colder periods, but also imply higher consumption of energy and associated resources, hence leading to higher GWP per FU.
- The comparison between ORP and CRP showed that both systems lead to different GWP impacts though they provide close annual productions. Under thermal regulation, the CRP has higher GWP impacts in warm periods (up to 61%) because of its needs of cooling energy whereas in cold periods it has lower GWP impact (down to -23%) since the reactor cover helps to keep heat in the culture which reduces the heating demand. Furthermore, without thermal regulation, ORP presented lower GWP impact than CRP, which due to the reactor cover, presents almost twice the GWP impact related to the ORP infrastructure.
- To balance the interest of maintaining high annual production whereas reducing GWP, the evaluation of specific scenarios showed that for both ORP and CRP culture modes, a trade-off was found for 8 months of culture (March to October) without thermal regulation in Nantes location (France).

Although the presented results are specific to Spirulina and the city of Nantes, the developed model can adapt to other strains and geographical locations by changing parameters related to microalgae and climate.

Complementary work to this study will include the implementation of the developed approach to assess and conceive cultivation facilities located in other latitudes around the world as well as industrial symbiosis where alternative resources are used to replace those significantly affecting the GWP impact (thermal energy, salts). Further studies will also evaluate the effect of the biomass cultivation conditions on downstream processes. Likewise, in the perspective of an industrial scale operation, a consequential LCA could be performed to account for the impacts on the feedstock suppliers.

# V. Acknowledgments

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