# Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale

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# Life cycle assessment of the production of the red anti-oxidant carotenoid astaxanthin by microalgae: From lab to pilot scale

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**Abstract**

The freshwater green microalga *Haematococcus pluvialis* is the richest source of natural astaxanthin. Astaxanthin is a high-value red carotenoid pigment commonly used in the food, feed and cosmetics industries due to its well-known antioxidant, anti-inflammatory and antitumour properties. This study assesses the environmental impacts associated with the production of natural astaxanthin from *H. pluvialis* at both lab and pilot scale. Closed airlift photobioreactors with artificial illumination, typically used for the production of high value products to avoid contamination risks and allow controlled lighting conditions, were considered. The study extends

from the production of the different inputs to the system to microalgal production, harvesting and further extraction of the carotenoid. The life cycle assessment was performed following the ISO 14040 and ten impact categories were considered in the study: abiotic depletion, acidification, eutrophication, global warming, ozone layer depletion, human toxicity, fresh water aquatic ecotoxicity, marine aquatic ecotoxicity, terrestrial ecotoxicity and photochemical oxidant formation.

According to the results, electricity requirements represented the major contributor to the environmental burdens among the activities involved in the production of astaxanthin. For the lab-scale process, the air supply and the production of chemicals and lab materials were also significant contributors in several categories. In the pilot-scale production, the relative environmental impacts were greatly reduced, partially due to changes implemented in the system as a result of lab-scale environmental assessment. However, the production of electricity still dominated the impacts in all categories, particularly due to the cultivation stage. For this reason, a sensitivity assessment was proposed in order to identify alternative photobioreactor configurations for astaxanthin production. Two of the evaluated options, based on the use of sunlight instead of artificial illumination, presented significant reductions of impact. However, the improvements observed in these cases were limited by the decrease in biomass productivity associated with sunlight culture systems. Therefore, a two flat-panel photobioreactor system with artificial illumination is proposed as a suitable option, allowing reductions between 62% and 79% of the impact depending on the considered category.

***Keywords*** Astaxanthin, Environmental assessment, *Haematococcus pluvialis*, Life Cycle Assessment, Life Cycle Inventory, microalgae, photobioreactor

## 1 Introduction

Microalgae are considered as a potential feedstock for the production of a wide diversity of compounds, ranging from value added products such as foodstuffs, chemicals, pharmaceuticals and nutraceuticals to next-generation biodiesel (Olaizola, 2003; Li et al., 2007; Sheehan et al., 1998). These organisms are the primary producers of organic matter in aquatic environments due to their photosynthetic activities (Suh et al., 2006). Under changing environmental conditions (e.g. nutrient deprivation, light limitation), microalgae can become stressed, causing them to overproduce some compounds of interest such as carotenoids (Aflalo et al., 2007; García-Malea et al., 2009; Shahid et al., 2013). As cell factories for the production of high value biomolecules, microalgae present numerous advantages such as i) the availability of cultivation on non-arable land; ii) the possibility of using wastewaters that provide the nutrients required for growth and, iii) the availability to modify the biochemical composition of the algal cells by varying the growth conditions (Stephenson et al., 2010). Even more, one of the most important and potential benefits of these organisms is the possibility of sequestrating $CO_2$ from streams derived from industrial processes (Munir et al., 2012; Olaizola, 2003).

Concerning the mechanisms used for microalgal cultivation, particular interest lies in the development of different cultivation technologies to improve productivity and yield (Brentner et al., 2011; Olaizola, 2003; Stephenson et al., 2010). In general, the cultivation systems can be classified in two main groups: i) open raceway ponds (ORPs) and ii) closed photobioreactors (PBRs). Important differences exist between both configurations: ORPs present higher losses by evaporation, larger requirements of water, higher risks of contamination, lower volumetric productivity, poor mixing and reduced temperature control in comparison with the PBRs. In contrast, they are less energy-intensive and are associated with lower levels of greenhouse gas

(GHG) emissions (Brentner et al., 2011; Jorquera et al., 2010). With regard to PBRs, they are closed systems with higher biomass yield but they are more expensive to build and operate than ORPs (Brentner et al., 2011; Jorquera et al., 2010; Stephenson et al., 2010).

Once the microalgae culture is grown, it is harvested. Depending on the product to be recovered, the next step typically entails reducing the water content of the microalgal biomass since low water content enhances the recovery of lipid soluble components and carotenoids (Brentner et al., 2011; Lardon et al., 2009). Different methods can be considered for this purpose: flocculation and settling, centrifugation, filtration or air flotation. The selection of the harvesting method will depend on factors such as energy requirement as well as microalgae cell characteristics: size and density (Brentner et al., 2011; Olaizola, 2003).

Finally, after harvesting, the compounds of interest are extracted by different methods. Commonly, organic solvents such as hexane or methanol (Kobayashi and Sakamoto, 1999; Stephenson et al., 2010) as well as supercritical fluids (Brentner et al., 2011) are used for extraction. Enzymatic extraction, still under development, could be a very interesting alternative to be established in the future specifically if the final product is intended for human consumption (Mercer and Armenta, 2011).

The freshwater green microalga *Haematococcus pluvialis* is the richest source of natural astaxanthin, a carotenoid (or pigment) commonly found in marine animals and traditionally used as a pigmentation source for fish aquaculture (Fábregas et al., 2001; García-Malea et al., 2005; Hata et al., 2001). Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione; $C_{40}H_{52}O_4$) is a high-value red carotenoid, which starts accumulating in the lipid vesicles of *H. pluvialis* during the transition between green vegetative cells and red aplanospores after exposure to stress conditions (Aflalo et al., 2007; Fábregas et al., 2001). However, astaxanthin production can be hampered by

the low cell growth rate, the sensitivity of the cells to hydrodynamic stress and changes in cell morphology under various environmental conditions (Hata et al., 2001). Due to its excellent antioxidant properties, astaxanthin has numerous applications; from its use as an additive in food and feed industries, nutraceuticals to cosmetics market (Koller et al., 2012). Recently, its anti-inflammatory and anti-cancer activities confirmed its importance in the medical sector (Aflalo et al., 2007; Guerin et al., 2003).

Many established large scale facilities produce natural astaxanthin from *H. pluvialis* (Algatechnologies Ltd.; BioReal AB; Cyanotech Corp.) despite the competition with the cheaper synthetic astaxanthin from petrochemical sources that dominates 95% of the current astaxanthin world market, estimated at US$250 million (Guerin et al., 2003; Lorenz and Cysewski, 2000; Murray et al. 2013; Olaizola, 2003). Consumer growing demand for natural products makes synthetic routes less desirable (Herrero et al., 2006; Lorenz and Cysewski, 2000), which justifies the considerable effort that is being paid on the promotion of biotechnological alternatives with environmental friendly production systems (Olaizola, 2003; Rodríguez-Sáiz et al., 2010). It is expected that in a short time, the production costs of the natural production process should be more competitive with synthetic astaxanthin after the optimisation of the production technology. In this sense, Li et al. (2011) have estimated a production cost of $718/kg natural astaxanthin in a conceptually designed plant of 900 kg astaxanthin per year, which is significantly lower to $1000/kg synthetic astaxanthin from companies such as DSM, BASF and NHU.

Furthermore, synthetic astaxanthin consists of a racemic mixture with a stereoisomeric ratio of 1:2:1 for the 3R,3´R/meso/3S,3´S isomers, whereas natural astaxanthin mainly corresponds to 3S,3´S isomer (Wang et al., 2008). This difference influences several properties related to the biological function of astaxanthin, such as the anti-oxidant potential or the shelf life, which

makes natural astaxanthin more valuable than the synthetic alternative in nutraceutical and pharmaceutical markets, reaching prices up to \$100,000 $kg^{-1}$ (Chen et al., 2007; Chew and Park, 2006; Olaizola, 2003; Spolaore et al., 2006).

Life Cycle Assessment (LCA) is a standardised methodology for addressing all the environmental concerns derived from the production process of a product by evaluating the potential environmental impacts associated with its whole life cycle chain (ISO 14040, 2006). To date, LCA has been usually applied to the evaluation of the environmental performance of algae-based biodiesel production (Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2010) and cofiring microalgae with coal (Kadam, 2002). Most of these studies were performed by extrapolation of lab and pilot-scale conditions due to the absence of data on biodiesel production from microalgae at industrial scale (Lardon et al., 2009). Although several LCA studies related to biotechnological processes as well as to the production of biologically active molecules have been already published (Jegannathan and Nielsen, 2013; Pietrzykowski et al., 2013), there are not available studies focused on the production of these biocompounds by microalgae. Therefore, to the best of our knowledge, this paper presents for the first time a detailed life cycle inventory and quantification of the related impacts of the production of high value natural astaxanthin from *Haematococcus pluvialis* using a photobioreactor with artificial illumination. The results highlight the most important environmental issues where future developments should pay special attention.

## 2 Materials and methods

### 2.1 Goal and scope definition

Similarly to previous works (Pietrzykowski et al., 2013), this study aims to perform a comparative assessment of the environmental impacts associated with the production of *H. pluvialis* astaxanthin for nutraceutical or pharmaceutical uses at both lab and pilot scale in airlift photobioreactors with artificial illumination. This dual approach will allow evaluating the differences between both perspectives, considering the influence of scale-up as well as the effectiveness of the changes introduced in the real pilot process after the lab-scale experiments. In a first stage, the environmental impacts associated with the operation of a 15 L tubular airlift photobioreactor were evaluated. In this case, astaxanthin was obtained as a pure compound after a conventional solvent extraction. The lab experiments regarding the cultivation of the microalga were carried out by the Bioengineering Group of the Earth and Life Institute at the University of Louvain (Belgium) while the extraction processes were developed by the Shannon Applied Biotechnology Centre at the Limerick Institute of Technology (Ireland).

Subsequently, a pilot-scale process was developed by the bio-technological company Algae Health (Ireland). This process used the information obtained from the laboratory system as a basis for the scale-up to a pilot process operated in two stages which was performed in consecutive 1000 L airlift photobioreactors. The target product from the pilot process was a nutraceutical oleoresin with a content of 10% astaxanthin.

In both stages, the study extends from the production of the different inputs to the system, to the cleaning of the reactor, the preparation of the culture medium, as well as microalgal cultivation, harvesting and final extraction of the carotenoid as a pure compound.

*2.1.1. Lab-scale process*

As mentioned, the main purpose of this study was to analyse the environmental effect of scale-up from laboratory to pilot-scale process for the production of *Haematococcus* astaxanthin extract from microalgae cultivated in photobioreactors. Lab-scale production in one step was considered initially in order to identify the main stages of the system and the most relevant hot spots that may also affect the pilot process.

The LCA study started with the selection of the functional unit, to which all inputs and outputs to the system are referred. For the lab-scale production, the functional unit chosen was 1 g of astaxanthin, equivalent to 1 batch of production.

The system for the production of astaxanthin from *Haematococcus pluvialis* was divided into five stages, which are described below: i) Cleaning of the reactor, ii) Preparation of the culture medium, iii) Cultivation of the microalga, iv) Harvesting and v) Extraction of the astaxanthin. **Figure 1** shows the different stages and processes that were included in the system boundaries.

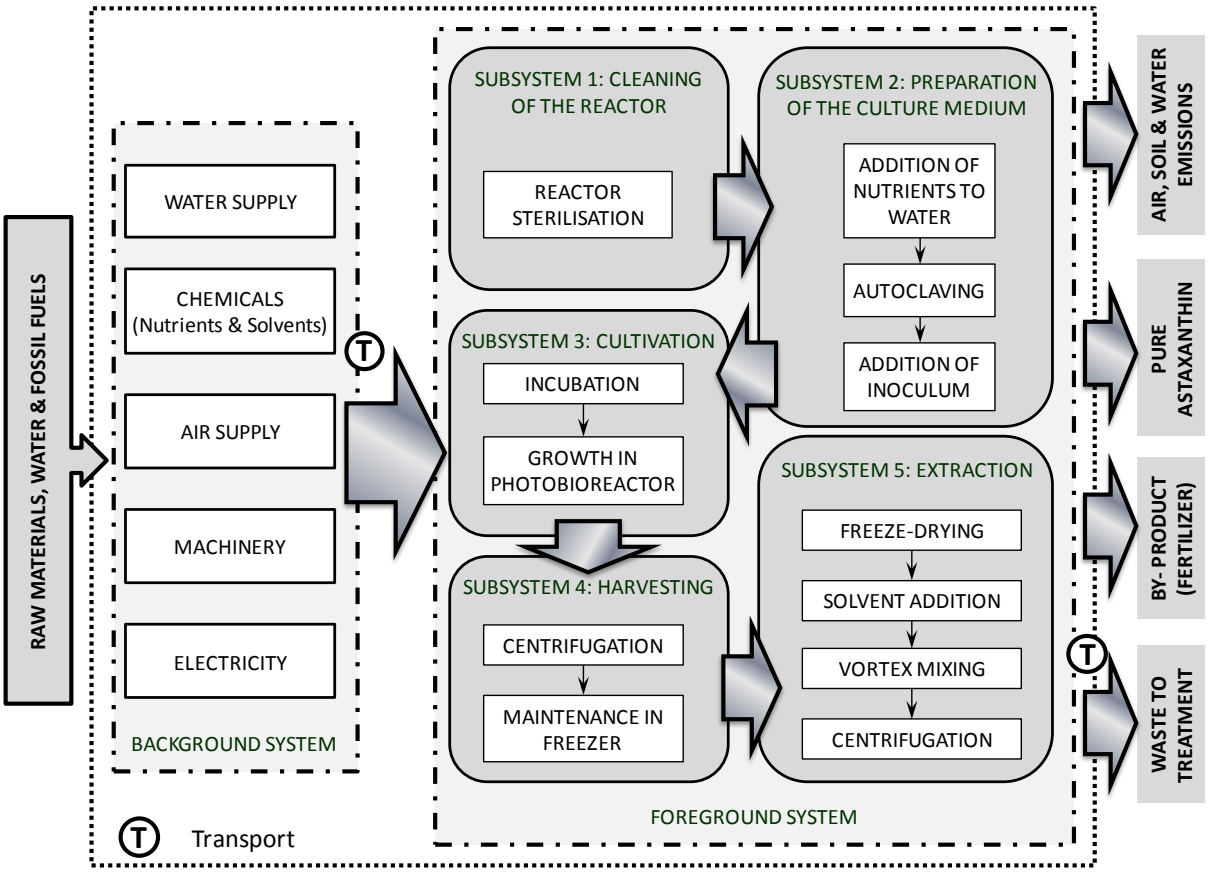


**Figure 1** System boundaries and process chain of the lab-scale case study: Production of astaxanthin from *Haematococcus pluvialis* in a 15L airlift photobioreactor.

*i) Cleaning of the reactor*

At lab-scale, the use of bleaching agents was considered to clean the reactor. For this purpose, 20 g of sodium hypochlorite, as well as 50 L of tap water and 30 L of sterile autoclaved water were required.

*ii) Preparation of the culture medium*

The culture medium comprised deionised water containing 0.75 g/L $NaNO_3$, 0.025 g/L $CaCl_2 \cdot 2H_2O$, 0.075 mM $MgSO_4 \cdot 7H_2O$, 0.025 g/L NaCl, 0.075 g/L $K_2HPO_4 \cdot 3H_2O$, 0.175 g/L $KH_2PO_4$, 0.0012 g/L vitamin B1, 0.00001 g/L vitamin B12 and trace metals (less than 0.005

g/L). This stage required the addition of nutrients in the specified amounts to deionised water, followed by the sterilisation of the culture medium in an autoclave, as well as the addition of the initial inoculum in 150 mL culture flasks under a sterile flow hood. Volumes of 1.5 L of inoculum and 13.5 L of culture medium were required for the start-up of the photobioreactor.

*iii) Cultivation*

Firstly, 150 mL cell cultures were statically incubated in flasks at 20ºC and 20 μmol photons·$m^{-2}$·$s^{-1}$ of light intensity from four fluorescent lights (15 W). Cells were subcultured and fresh medium was added in order to increase the cell culture density from 0.3 dry weight ($g_{DW}$)·$L^{-1}$ to 2 $g_{DW}$·$L^{-1}$. Once the required density was reached, the inoculum was added to the prepared medium in the photobioreactor.

The lab-scale photobioreactor consisted of a polyvinyl chloride (PVC) tubular airlift photobioreactor with a volume of 15 L. The reactor was illuminated by six fluorescent bulbs of 36 W and aerated by 1.5 L·$min^{-1}$ of compressed air enriched with 0.5% $CO_2$ in the feed gas. In this case, 30 g of dried biomass were produced in one batch after 14 days of operation in a single stage, with an astaxanthin content of 4%.

*iv) Harvesting*

The biomass produced was initially harvested by centrifugation with an efficiency of 95%. A volume reduction of 97% was obtained, with final moisture of 94%. The resulting biomass was kept in a freezer before being freeze-dried to 2% moisture.

*v) Extraction*

The lab-scale separation process consisted of a conventional solvent extraction with dimethyl sulfoxide (DMSO). After DMSO addition, the mixture was heated to 55ºC and vortexed before separating the pigment phase by centrifugation. Finally, 1 g astaxanthin was obtained with a

purity of 95%. As in the case of other marine organisms (Spångberg et al., 2013), the algal residue was considered as a fertiliser due to its content in nitrogen and phosphorous. However, it is important to remark here that this residue contained high-value added components with antioxidant and antimicrobial activities that may have more specialised potential applications.

### *2.1.2. Pilot-scale process*

After the optimisation of operational parameters, a two-stage pilot system was designed. The process consisted of a first growth stage with nutrient excess followed by a second stress stage limited in phosphate and nitrate. In both stages, the excess culture medium was recirculated, so at least five cultures could be performed with the water initially added. Therefore, the selected functional unit was 800 g of astaxanthin, corresponding to a complete cycle of five cultures.

In order to obtain applicable conclusions from the lab-scale study, the pilot system was divided into the same five stages as the lab production of *Haematococcus* astaxanthin, as shown in **Figure 2**. The deviations from the original lab process are detailed below.

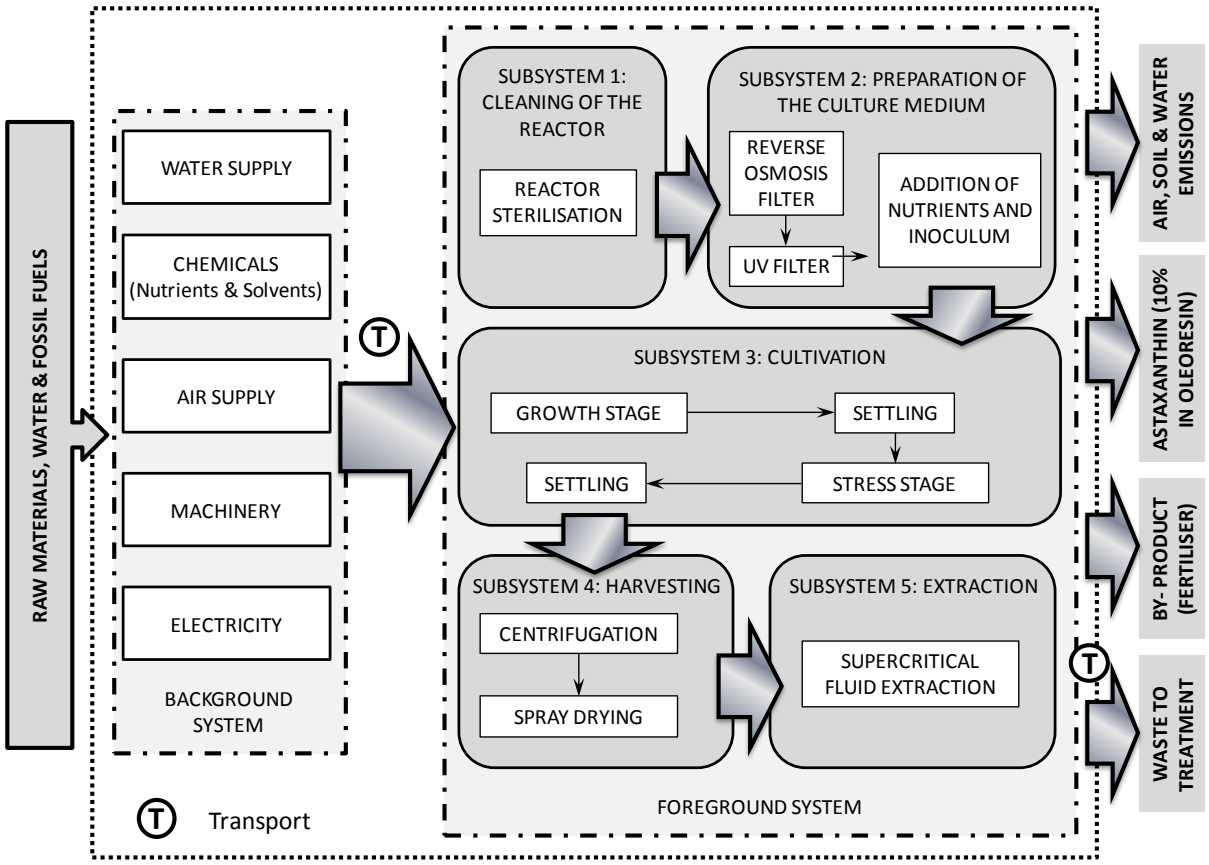


**Figure 2** System boundaries and process chain of the pilot-scale case study: Production of astaxanthin from *Haematococcus pluvialis* in a two-stage process with 1000 L internally illuminated airlift photobioreactor.

*i) Cleaning of the reactor*

In the case of the pilot process, two options were evaluated for the cleaning and sterilisation stage. Firstly, the use of bleaching agents was considered. Another possibility was assessed, consisting of the circulation of ozonised water through the reactor for a 4-h period, which seems to be a more appropriate option for a commercial scale production.

*ii) Preparation of the culture medium*

The culture medium for the pilot process was prepared with river and rain water, previously purified by reverse osmosis and UV filter to remove undesired salts and microorganisms. As the cultivation was carried out in two stages, namely growth and stress stage, two cultivation mediums were prepared. In the medium for the growth stage, the added nutrients were 0.875 g/ L $NaNO_3$, 0.1975 g/L $K_2HPO_4$, 0.0875 g/L $KH_2PO_4$, 0.0305 $CaCl_2$ g/L , 0.141 g/L $MgSO_4$, 0.0125 NaCl, 0.004 g/L citric acid, 0.05 g/L $Na_2CO_3$, 0.00275 g/L disodium ethylenediaminetetraacetate (EDTA), 0.00143 $H_3BO_3$ and trace elements (less than 0.001 g/L). The culture medium used for the stress stage was equivalent to the previous one, except for the absence of $K_2HPO_4$ and $KH_2PO_4$, as well as for the concentration of $NaNO_3$, which was reduced to 0.0875 g/L.

*iii) Cultivation*

In the first stage, the microalga was grown for 8 days in a 1000 L airlift photobioreactor with an excess of nutrients. The reactor was internally illuminated with a 16:8 regime (600 W) and continuously aerated (50 W, 24 h). Afterwards, 50% of the cell culture was taken to an analogous reactor and microalgal cells were allowed to settle, enabling the drainage of 96% of the water, that could be recirculated to the original tank. In the second tank, a stress medium with limiting phosphate and nitrate nutrients was added, inducing astaxanthin accumulation. This stressing cycle required 1200 W for the illumination, as well as 50 W for the agitation motor and 50 W for the aeration pump at a continuous rate during 8 days. At the end of the period, the microalga had turned red and accumulated 4-5% astaxanthin. After settling of the culture broth, approximately 80% water was recovered and recirculated. The remaining 20% water was then poured off and sent to harvesting stage.

*iv) Harvesting*

As in the lab-scale process, the biomass was harvested by centrifugation with approximately 95% efficiency. However, in this case a settling step was carried out before centrifugation to preconcentrate the biomass. Therefore, the starting moisture for the centrifugation was lower than that of the lab-scale process, and consequently a lower moisture of 80% was obtained in the pilot process. After spray-drying the algal paste, the moisture content was reduced to 5%.

*v) Extraction*

The lab-scale separation process consisted of a conventional extraction with DMSO. However, this method is not suitable for the production of astaxanthin used in food or pharmaceutical industries due to the DMSO residue (Ni et al., 2007). Therefore, a supercritical $CO_2$ extraction was chosen to isolate astaxanthin from the algal paste obtained in the pilot-scale process. To do so, the cells were mixed with a dispersing and drying agent prior extraction. Both fish and vegetable oils could be used as a co-solvent. In this case, fish waste oil was employed, in a ratio of 25% weight of sample.

Although the final product of the pilot process is an oleoresin with 10% astaxanthin, the final processing was excluded from the system boundaries to make the results comparable to those of lab-scale process. Thus, the production of pure astaxanthin (95%) was assessed as the final product, and the algal residue was considered as a fertiliser.

## 2.2 Inventory analysis, data quality and simplifications

The Life Cycle Inventory (LCI) data for the foreground system (i.e. chemicals and electricity consumptions as well as transport distances) consisted of average data obtained by on-site measurements. Concerning water emissions, they were calculated assuming that the nutrients

supplied in the culture medium which are not consumed during the algae growth, are directly discharged to water. An identical assumption was made for air emissions.

Concerning the background system, the corresponding inventory data for the production of all the inputs to the system were taken from Ecoinvent database, except from metal components, that were taken from IDEMAT (2001). These inputs include the production of the different chemicals required for the preparation of the culture medium, the electricity used in the different production stages, the distribution of inputs up to the lab gate, lab ware supplies and equipment (flasks, photobioreactor, fluorescent tubes, electronic devices) and waste disposal. A detailed description of the corresponding database reports considered is shown in **Table 1~~2~~**. For the equipments, different service lives were considered, according to manufacturers' specifications. An average transport distance of 800 and 600 km within continental Europe was considered for chemicals and materials, respectively, with an average sea distance of 1,400 km from the continental Europe to Ireland in the pilot-scale process. Waste transport distance was estimated around 50 km. Disposal in sanitary landfill was considered for all plastic waste, whereas steel components and lamps were sent to either inert landfills or specific waste treatment. Incineration was considered for the filter membrane (polyamide).

**Table 1** Summary of data sources.

| | | |
|---|---|---|
| **Energy** | Electricity (Belgian electricity profile) | Ecoinvent database (Dones et al. 2007) |
| | Electricity (Irish electricity profile) | |
| **Chemicals** | $NaNO_3$[†] | Ecoinvent database (Althaus et al. 2007) |
| | $CaCl_2$ | |
| | $MgSO_4$ | |
| | NaCl | |
| | $K_2HPO_4$ | |
| | $KH_2PO_4$ | |
| | $C_{12}H_{17}ClN_4OS \cdot HCl$ | |
| | $C_{63}H_{88}CoN_{14}O_{14}P$ | |
| | $C_{10}H_{16}N_2O_8$ | |
| | $FeCl_3$ | |
| | $MnCl_2$ | |
| | $ZnCl_2$ | |
| | $CoCl_2$ | |
| | $Na_2MoO_4$ | |
| | $C_6H_8O_7$ | |
| | $C_6H_{5+4y}Fe_xN_yO_7$ | |
| | $H_3BO_3$ | |
| | $ZnSO_4$ | |
| | $CuSO_4$ | |
| | $Co(NO_3)_2$ | |
| | $CO_2$ | |
| | $C_2H_6OS$ (DMSO) | |
| | NaClO | |
| | Deionised water | |
| | Tap water | |
| | $Na_2CO_3$ | Ecoinvent database (Sutter 2007) |
| **Air supply** | Compressed air | Ecoinvent database (Steiner and Frischnecht 2007) |
| | Carbon dioxide | Ecoinvent database (Althaus et al. 2007) |
| **Materials** | PVC | Ecoinvent database (Hischier 2007) |
| | Polystyrene | |
| | High density polyethylene | |
| | Polyethylene terephthalate | |
| | Gro-lux fluorescent tubes (36 W) | Ecoinvent database (Hischier et al. 2007) |
| | Stainless steel | IDEMAT (2001) |
| | Galvanised steel | |
| **Transport** | Truck 3.5-7.5 t, Euro 4 | Ecoinvent database (Spielmann et al. 2007) |
| | Freight ship | |
| **Waste treatment** | Sanitary landfill | Ecoinvent database (Doka 2007) |

[†] Synthetic route - UNIDO/IFDC (1998) and Oak Ridge National Laboratory (1994)

With regard to $NaNO_3$ production, this process is not defined in the Ecoinvent database. Therefore, the considered inventory data correspond to the synthetic process described in UNIDO/IFDC (1998). The method, developed by GIAP, consists of an oxidation of ammonia in the presence of platinum catalyst followed by the absorption of the nitrogen oxides produced in an aqueous solution of sodium carbonate and the separation of sodium nitrate and sodium nitrite. Finally, nitric acid is added to convert sodium nitrite to sodium nitrate. Sodium nitrate is separated from the solution and dried in a rotary dryer. Inventory data for the raw materials were taken from Ecoinvent database, whereas energy requirements from Bhat et al. (1994) were considered.

Fish oil, required for the supercritical extraction stage in the pilot-scale process, is not available in the Ecoinvent database. Fish oil is a by-product of fisheries, obtained from the discarded fraction of marine fish such as mackerel, salmon, tuna and cod (Lin and Li, 2009). In this case, the inventory data from Iribarren et al. (2012) were considered.

In this study, no allocation procedure was required since algae cultivation was only focused on astaxanthin production. Therefore, all the environmental burdens were allocated to the amount of astaxanthin produced. The separated biomass residue was considered as a potential fertiliser and the corresponding contents in nitrogen and phosphorous were calculated according to Mulbry et al. (2005). Thus, a nitrogen content of 7% in algal biomass was considered, with 30% of total nitrogen as plant available nitrogen. Regarding phosphorus, it was assumed a content of 1% present in biomass, with 60% as plant available phosphorus. Once the fertiliser potential was estimated, the equivalent amount of a typical fertiliser (ammonium sulphate as N source and diammonium phosphate as P source) was considered in the model as avoided product, which resulted in negative impacts that were subtracted from the environmental burdens.

The global inventories of lab and pilot-scale processes are shown in **Table 2** and **Table 3**.

**Table 2** Global inventory for the lab-scale production of astaxanthin from *Haematococcus pluvialis* in a 15 L tubular airlift photobioreactor (functional unit: 1 g astaxanthin)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | **Materials** | |
| ***Cleaning of the reactor*** | | ***Extraction*** | |
| Deonised water | 28.27 L | Dimethyl sulfoxide (DMSO) | 2.63 L |
| Tap water | 47.11 L | Stainless steel | 0.31 g |
| Sodium hypochlorite (NaClO) | 18.84 g | Cast metal | 0.94 g |
| Stainless steel | 4.70 g | Galvanised steel | 8.16 g |
| ***Preparation of the culture medium*** | | Anodised aluminium | 0.22 g |
| Deonised water | 14.13 L | Polycarbonate | 211.05 g |
| $NaNO_3$ | 10.571 g | | |
| $CaCl_2{\cdot}2H_2O$ | 0.352 g | | |
| $MgSO_4.7H_2O$ | 1.057 g | **Energy** | |
| NaCl | 0.352 g | ***Cleaning of the reactor*** | |
| $K_2HPO_4.3H_2O$ | 1.057 g | Autoclaving | 1.11 kWh |
| $KH_2PO_4$ | 2.466 g | ***Preparation of the culture medium*** | |
| $C_{12}H_{17}ClN_4OS{\cdot}HCl$ | 0.0169 g | Autoclaving | 0.78 kWh |
| $C_{63}H_{88}CoN_{14}O_{14}P$ | 0.0001 g | Addition of inoculum in laminar flow hood | 0.19 kWh |
| $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ | 0.0634 g | ***Cultivation*** | |
| $FeCl_3$ | 0.0049 g | Incubation (excluding lights) | 40.70 kWh |
| $MnCl_2$ | 0.0035 g | Lighting in incubation stage | 9.50 kWh |
| $ZnCl_2$ | 0.0004 g | Lighting in the photobioreactor | 68.38 kWh |
| $CoCl_2{\cdot}6H_2O$ | 0.0002 g | Air blowing | 3.73 kWh |
| $Na_2MoO_4.2H_2O$ | 0.0003 g | ***Harvesting*** | |
| Stainless steel | 26.14 g | Centrifugation | 10.99 kWh |
| Polystyrene (PS) | 3.37 kg | Freezer | 5.65 kWh |
| High density polyethylene (HDPE) | 124.37 g | Freeze-drying | 2.26 kWh |
| Polyethylene terephthalate (PET) | 0.96 g | ***Extraction*** | |
| ***Cultivation*** | | Heating of the solvent | 0.39 kWh |
| Compressed air (enriched 0.5% $CO_2$) | 67.91 kg | Vortex mixing | 0.05 kWh |
| Fluorescent lamps (15 W) | 3.04 g | Centrifugation | 1.28 kWh |
| Polyurethane foam | 34.26 g | | |
| Galvanised steel | 79.94 g | | |
| Gro-lux fluorescent tubes (36 W) | 16.15 g | **Transport** | |
| Polyvinyl chloride (PVC) | 40.47 g | Truck, 3.5-7.5 t, Euro 4 (Chemicals) | 2.344 tkm |
| ***Harvesting*** | | Truck, 3.5-7.5 t, Euro 4 (Materials) | 3.134 tkm |
| Distilled water | 4.71 L | Truck, 3.5-7.5 t, Euro 4 (Waste) | 0.261 tkm |
| Polypropylene (PP) | 819.72 g | | |
| Galvanised steel | 32.96 g | | |
| Anodised aluminium | 0.85 g | | |
| Polycarbonate | 422.11 g | | |
| Polyurethane foam | 3.00 g | | |
| Stainless steel | 20.70 g | | |
| **INPUTS from ENVIRONMENT** | | | |
| **Materials** | | | |
| Biomass | 0.424 g | | |
| **OUTPUTS to TECHNOSPHERE** | | **OUTPUTS to ENVIRONMENT** | |
| **Product** | | **Air emissions** | |
| *Haematococcus* astaxanthin | 1 g | Air (excluding $CO_2$) | 67.39 kg |
| **Avoided product**[1] | | $CO_2$ | 0.48 kg |
| Nitrogen-rich fertiliser | 0.573 g | **Water emissions** | |
| Phosphorous-rich fertiliser | 0.375 g | Water effluent | 94.22 L |
| **Waste treatment** | | NaClO | 18.84 g |
| Steel, to inert landfill | 173.847 g | $NaNO_3$ | 0.4770 g |
| Polystyrene, to sanitary landfill | 3.373 kg | $CaCl_2{\cdot}2H_2O$ | 0.0159 g |

| | | | |
|---|---|---|---|
| Polyethylene, to sanitary landfill | 124.371 g | $MgSO_4.7H_2O$ | 0.0477 g |
| Polyethylene terephthalate | 0.964 g | NaCl | 0.0159 g |
| Fluorescent lamps, to specific treatment for electronics wastes | 19.185 g | $K_2HPO_4.3H_2O$ | 0.0477 g |
| | | $KH_2PO_4$ | 0.1113 g |
| Polyurethane foam, to specific waste treatment | 37.26 g | $C_{12}H_{17}ClN_4OS{\cdot}HCl$ | 0.0008 g |
| Polyvinyl chloride, to sanitary landfill | 40.469 g | $C_{63}H_{88}CoN_{14}O_{14}P$ | 0.00001 g |
| Polypropylene, to sanitary landfill | 819.718 g | $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ | 0.0029 g |
| Aluminium, to sanitary landfill | 1.067 g | $FeCl_3$ | 0.0002 g |
| Polycarbonate, to sanitary landfill | 633.162 g | $MnCl_2$ | 0.0002 g |
| | | $ZnCl_2$ | 0.00002 g |
| | | $CoCl_2{\cdot}6H_2O$ | 0.00001 g |
| | | $Na_2MoO_4.2H_2O$ | 0.00002 g |
| | | DMSO | 2.6316 L |

**Table 3** Global inventory for the production of astaxanthin from *Haematococcus pluvialis* in a two-stage process with 1000 L stirred-tank photobioreactors in series (functional unit: 800 g carotenoid).

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | **Materials** | |
| ***Cleaning of the reactor*** | | ***Extraction*** | |
| OPTION 1 | | Drying agent (pelletised diatomaceous earth) | 35.46 kg |
| Tap water | 4.009 $m^3$ | Co-solvent (fish/vegetable oil) | 4.67 kg |
| NaClO | 4.009 kg | Stainless steel | 0.55 kg |
| OPTION 2 | | | |
| Stainless steel | 0.200 kg | **Energy** | |
| ***Preparation of the culture medium*** | | ***Cleaning of the reactor (OPTION 2)*** | |
| $NaNO_3$ | 4.4651 kg | Reactor sterilisation with ozonised water | 0.12 kWh |
| $K_2HPO_4$ | 0.9121 kg | ***Preparation of the culture medium*** | |
| $KH_2PO_4$ | 0.4041 kg | Reverse osmosis filtration | 7.71 kWh |
| $CaCl_2$ | 0.2914 kg | UV filtration | 0.35 kWh |
| $MgSO_4$ | 1.3473 kg | ***Cultivation*** | |
| NaCl | 0.1194 kg | Lighting in the photobioreactor | 1,539.43 kWh |
| $C_6H_8O_7$ | 0.0287 kg | Air blowing | 96.21 kWh |
| $C_6H_{5+4y}Fe_xN_yO_7$ | 0.0287 kg | Agitation | 96.21 kWh |
| $Na_2CO_3$ | 0.4778 kg | ***Harvesting*** | |
| $C_{10}H_{16}N_2O_8$ | 0.0263 kg | Centrifugation | 1.50 kWh |
| $H_3BO_3$ | 0.0137 kg | Spray drying | 82.70 kWh |
| $ZnSO_4$ | 0.0011 kg | ***Extraction*** | |
| $CuSO_4$ | 0.0004 kg | Supercritical $CO_2$ extraction | 158.25 kWh |
| $Co(NO_3)_2$ | 0.0002 kg | | |
| $FeCl_3$ | 0.0028 kg | **Transport** | |
| $ZnCl_2$ | 0.0001 kg | OPTION 1 | |
| $CoCl_2$ | 0.0001 kg | Truck, 3.5-7.5 t, Euro 4 (Chemicals) | 41.87 tkm |
| $MnCl_2$ | 0.0098 kg | Truck, 3.5-7.5 t, Euro 4 (Materials) | 8.03 tkm |
| $Na_2MoO_4$ | 0.0012 kg | Truck, 3.5-7.5 t, Euro 4 (Waste) | 2.44 tkm |
| Stainless steel | 0.3446 kg | Ship (Chemicals) | 73.27 tkm |
| Polyvinyl chloride (PVC) | 0.0213 kg | Ship (Materials) | 18.73 tkm |
| UV lamps | 0.0175 kg | OPTION 2 | |
| Polyamide | 0.1169 kg | Truck, 3.5-7.5 t, Euro 4 (Chemicals) | 38.60 tkm |
| ***Cultivation*** | | Truck, 3.5-7.5 t, Euro 4 (Materials) | 8.15 tkm |
| Stainless steel | 8.36 kg | Truck, 3.5-7.5 t, Euro 4 (Waste) | 2.45 tkm |
| Reactor lamps | 0.13 kg | Ship (Chemicals) | 67.55 tkm |
| ***Harvesting*** | | Ship (Materials) | 19.01 tkm |
| Stainless steel | 3.84 kg | | |
| **INPUTS from ENVIRONMENT** | | | |
| **Materials** | | **Materials** | |
| Biomass | 0.017 kg | Air (excluding $CO_2$) | 434.72 t |
| River/rain water | 2.786 $m^3$ | $CO_2$ | 0.26 t |

| **OUTPUTS to TECHNOSPHERE** | | **OUTPUTS to ENVIRONMENT** | |
|---|---|---|---|
| **Product** | | **Water emissions** | |
| *Haematococcus* astaxanthin | 800 g | $NaNO_3$ | 0.0891 kg |
| **Avoided product**[1] | | $K_2HPO_4$ | 0.0194 kg |
| Nitrogen-rich fertiliser | 0.431 kg | $KH_2PO_4$ | 0.0086 kg |
| Phosphorous-rich fertiliser | 0.282 kg | $CaCl_2$ | 0.0041 kg |
| **Waste treatment** | | $MgSO_4$ | 0.0189 kg |
| Steel, to inert landfill (OPTION 1) | 13.09 kg | NaCl | 0.0017 kg |
| Steel, to inert landfill (OPTION 2) | 13.29 kg | $C_6H_8O_7$ | 0.0004 kg |
| Polyvinyl chloride, to sanitary landfill | 0.02 kg | $C_6H_{5+4y}Fe_xN_yO_7$ | 0.0004 kg |
| Fluorescent lamps, to specific treatment for electronics wastes | 0.15 kg | $Na_2CO_3$ | 0.0067 kg |
| | | $C_{10}H_{16}N_2O_8$ | 0.0004 kg |
| Textiles, to municipal incineration | 0.12 kg | $H_3BO_3$ | 0.0002 kg |
| Diatomaceous earth, to inert landfill | 35.46 kg | $ZnSO_4$ | 0.00001 kg |
| | | $CuSO_4$ | 0.000005 kg |
| | | $Co(NO_3)_2$ | 0.000003 kg |
| | | $FeCl_3$ | 0.000039 kg |
| **OUTPUTS to ENVIRONMENT** | | $ZnCl_2$ | 0.000002 kg |
| **Air emissions** | | $CoCl_2$ | 0.000001 kg |
| Air (excluding $CO_2$) | 434.72 t | $MnCl_2$ | 0.000138 kg |
| $CO_2$ | 0.24 t | $Na_2MoO_4$ | 0.000016 kg |
| | | OPTION 1 | |
| | | Water effluent | 6.79 $m^3$ |
| | | NaClO | 4.009 kg |
| | | OPTION 2 | |
| | | Water effluent | 2.79 $m^3$ |

## 2.3 Life Cycle Impact Assessment

Regarding the steps defined within the life cycle impact assessment, classification and characterisation stages were undertaken here (ISO 14040, 2006). Normalisation and weighting were not conducted as these optional (and, to some extent, subjective) elements were not considered to provide additional, robust information for the objectives established in this study. The characterisation factors reported by the Centre of Environmental Science of Leiden University (CML 2001 method) were used (Guinée et al., 2001). The impact potentials (or impact categories) evaluated according to the CML method were: abiotic depletion (ADP), acidification (AP), eutrophication (EP), global warming (GWP), ozone layer depletion (ODP), human toxicity (HTP), freshwater aquatic ecotoxicity (FEP), marine aquatic ecotoxicity (MEP), terrestrial ecotoxicity (TEP) and photochemical oxidants formation (POFP). The software

SimaPro 7.3 was used for the computational implementation of the inventories (Goedkoop et al., 2008).

## 3 Results and discussion

### 3.1 Lab-scale environmental profile

The characterisation results of the astaxanthin production process are shown in ~~Table 3~~ **Table 4** for the complete production process (cradle-to-gate perspective) at laboratory scale. A breakdown of the contribution of the production stages is depicted in **Figure 3** in order to identify the most relevant one for the selected environmental impact categories.

**Table 4** Impact assessment results (characterisation step) associated to the lab-scale production of 1 g of *Haematococcus* astaxanthin.

| Impact category | Unit | Value |
|---|---|---|
| ADP | kg $Sb_{eq}$ | 0.716 |
| AP | kg $SO_{2\ eq}$ | 0.447 |
| EP | kg $PO_4^{-3}{}_{eq}$ | 0.157 |
| GWP | kg $CO_{2\ eq}$ | 87.2 |
| ODP | g CFC-11 $_{eq}$ | 0.005 |
| HTP | kg 1,4-DB $_{eq}$ | 48.6 |
| FEP | kg 1,4-DB $_{eq}$ | 33.8 |
| MEP | kg 1,4-DB $_{eq}$ | 20.2 |
| TEP | g 1,4-DB $_{eq}$ | 4.72 |
| POFP | g $C_2H_{4\ eq}$ | 15.4 |

According to **Figure 3**, the contribution from the cultivation stage is the main factor responsible for the environmental burdens derived from the production of astaxanthin with remarkable contributions of more than 44% to all impact categories under assessment. Amongst the less

contributing stages, the preparation of the medium plays a significant role in categories such as ADP, EP, GWP or POFP, whereas the extraction is only relevant in terms of AP and ODP, and harvesting mainly contributes to ecotoxicity categories.

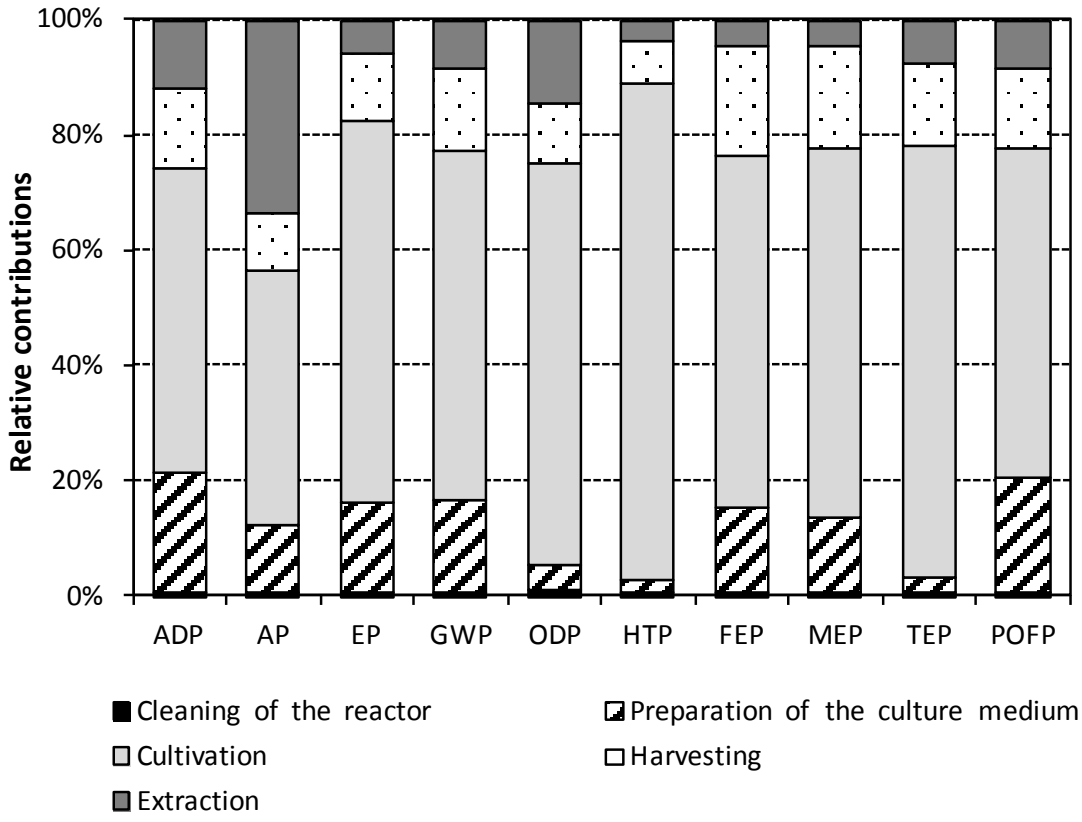


**Figure 3** Relative contribution (in %) per stage of lab-scale process to each impact category.

The contributing processes to each category are discussed below per impact category and stage and the environmental contributions are broken down in **Figure 4**.

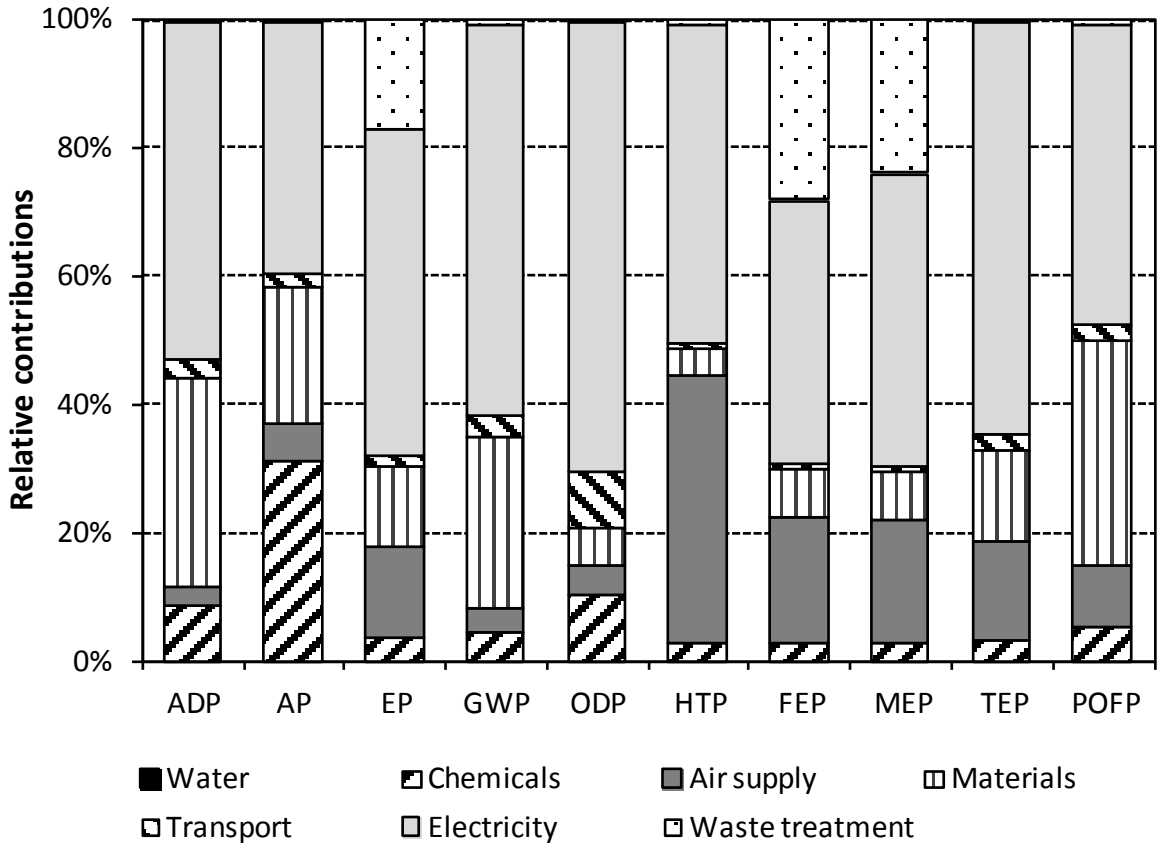


**Figure 4** Relative contribution (in %) per involved activity of lab-scale process to each impact category

*Abiotic depletion potential*. The production of the electricity requirements was responsible for 53% of contributions to ADP, especially due to the consumption of electricity in the PBR (50% of total electricity, considering lighting and air blower). It shall be remarked here that the Belgian electricity profile depends considerably on fossil fuels, which was reflected on the ADP results. According to **Figure 4**, the second most important factor responsible for abiotic depletion was the production of materials with a remarkable contribution of 33%, especially due to the production of polystyrene.

*Acidification potential*. The production of electricity and chemicals were the main contributors to AP (39% and 31% respectively). $SO_2$ emissions from coal power plants were the main responsible for the impact derived from the production of electricity. In the case of chemicals, the impact is mainly due to the manufacture of DMSO (99% of total AP), in which acidifying emissions of hydrogen sulphide are generated.

*Eutrophication potential*. The production of electricity requirements was again the main factor for eutrophying emissions and its contribution to EP was 51% of the total. In order of relevance, it was followed by waste treatment (17%) and aeration (14%).

*Global warming potential*. GHG emissions were mainly derived from the production of electricity requirements (61%). According to Figure 3, the production of materials for the equipment and lab ware supplies contributed to 27% of GHG emissions. The main greenhouse gas responsible for the GWP was fossil $CO_2$ emissions (90%), especially derived from electricity production.

*Ozone layer depletion potential*. This category was affected up to 70% by the production of electricity, mainly due to its dependence on fossil fuels. The production of chemicals and the transport of inputs and waste are the most significant secondary contributors, with 10% and 8% of emissions, respectively. The main contributing substance was Halon 1211 (43%) emitted to air during the transport of natural gas in the production of electricity and DMSO.

*Human toxicity*. As in the previous categories, the production of electricity was the main factor which contributed to HTP with a ratio of 50%, followed by the compressed air required in the PBR (42%). This is caused by the emissions to air of arsenic (33%), chromium VI (27%) and polycyclic aromatic hydrocarbons (16%).

*Ecotoxicity potentials.* The contributing processes involved in these three impact categories follow the same trend as in other categories, where the production of the electricity was the major environmental key factor. Contributions in terms of FEP (41%) and MEP (45%) are associated with the emissions of vanadium, nickel and beryllium to water, especially coming from the disposal of materials in landfills. In the case of TEP, the impacts are mainly due to mercury (41%) to the air derived from the use of coal for electricity generation and chromium VI (32%) to the soil from the distribution network. Waste treatment showed a remarkable contribution in terms of FEP (28%) and MEP (24%), followed by air supply (20% and 19% to FEP and MEP, respectively).

*Photochemical oxidants formation potential.* The 47% of contributions to this category were due to the electricity consumption in the astaxanthin production process. The second most important factor was the production of materials for the equipments and lab ware supplies (35%). The main contributing substances to POFP were $SO_2$ (59%) and CO (15%).

### 3.2 Pilot-scale environmental profile

The characterisation results of the astaxanthin process at pilot-scale are shown in **Table 5** for the two options evaluated for the cleaning and sterilisation stage. In order to analyse the improvement of the pilot process in contrast with the information obtained at lab-scale, the results of the lab process are also presented in terms of an identical functional unit. It must be remarked, however, that this would not be a realistic functional unit for a lab process and, thus, it is here used for comparative purposes, exclusively.

**Table 5** Impact assessment results (characterisation step) associated to the pilot-scale production of 800 g of *Haematococcus* astaxanthin with two evaluated options for the cleaning stage in comparison with the corresponding impacts of the lab-scale process.

| Impact category | Unit | Lab-scale process | Chemical disinfection | Ozone sterilisation |
|---|---|---|---|---|
| ADP | kg $Sb_{eq}$ | 572.89 | 13.6 | 13.6 |
| AP | kg $SO_{2\ eq}$ | 355.76 | 12.1 | 12.1 |
| EP | kg $PO_4^{-3}{}_{eq}$ | 125.78 | 1.90 | 1.88 |
| GWP | t $CO_{2\ eq}$ | 69.75 | 1.86 | 1.86 |
| ODP | g CFC-11 $_{eq}$ | 3.83 | 0.130 | 0.129 |
| HTP | kg 1,4-DB $_{eq}$ | 38,886.69 | 321 | 319 |
| FEP | kg 1,4-DB $_{eq}$ | 27,029.44 | 261 | 259 |
| MEP | kg 1,4-DB $_{eq}$ | 16,166.48 | 190 | 188 |
| TEP | kg 1,4-DB $_{eq}$ | 3.77 | 0.088 | 0.087 |
| POFP | kg $C_2H_{4\ eq}$ | 12.32 | 0.490 | 0.490 |

The obtained values show that there is no significant difference between the environmental impacts of both pilot-scale alternatives in all the assessed categories, although a slight improvement is observed for the case of ozone sterilisation. As the results differ in less than 1.5% for all impact categories, the following analysed contributions will be referred to the second option: ozone sterilisation, as this seems the most realistic scenario for a commercial scale plant.

Regarding the improvement with respect to the lab-scale process, environmental impacts of both pilot options were found between 25 and 122 times lower than the corresponding lab-scale impacts. According to the results, the most remarkable reductions correspond to the toxicity potentials, which were the categories with higher contribution of air supply and waste treatment activities in the lab-scale process. It must be highlighted that compressed enriched air used in the lab process was substituted by air from the environment as $CO_2$ source in the pilot system. In addition, toxicities were related to relatively important contributions of the harvesting stage, which was also modified in the pilot process.

According to **Figure 5**, the contribution from the cultivation stage is again the main factor responsible for the environmental burdens derived from the production of astaxanthin with higher relative contributions than those of lab-scale process (more than 75% to all impact categories under assessment). Even so, impact reductions up to 95% were observed in all the evaluated categories with respect to lab-scale process, mainly related to the substitution of the cultivation in a single reactor by the two-stage cultivation process.

In the case of other stages, such as preparation of the culture medium or extraction, the relative contributions with reference to the total impacts of the pilot process have moderately decreased, probably due to the optimisation of the process and the different technologies applied. Particularly, supercritical technology has already been highlighted as a less energy-consuming separation alternative than organic solvent extraction (Aresta et al., 2004; 2005). Regarding this issue, Brentner et al. (2011) found an energy demand 4.5 times higher for hexane extraction than the corresponding value for supercritical $CO_2$, whereas supercritical methanol extraction allowed even reduced energy consumption, 5% lower than that of supercritical $CO_2$. In this case, the extraction stage of the pilot-scale process shows impact reductions of about 50 times lower than the lab process in most categories. The improvement is even more noticeable in the case of AP (1% of impact in pilot process with respect to total contribution of the lab-scale extraction). The main reason for this finding may be the removal of DMSO, associated with acidifying emissions of hydrogen sulphide during the production process.

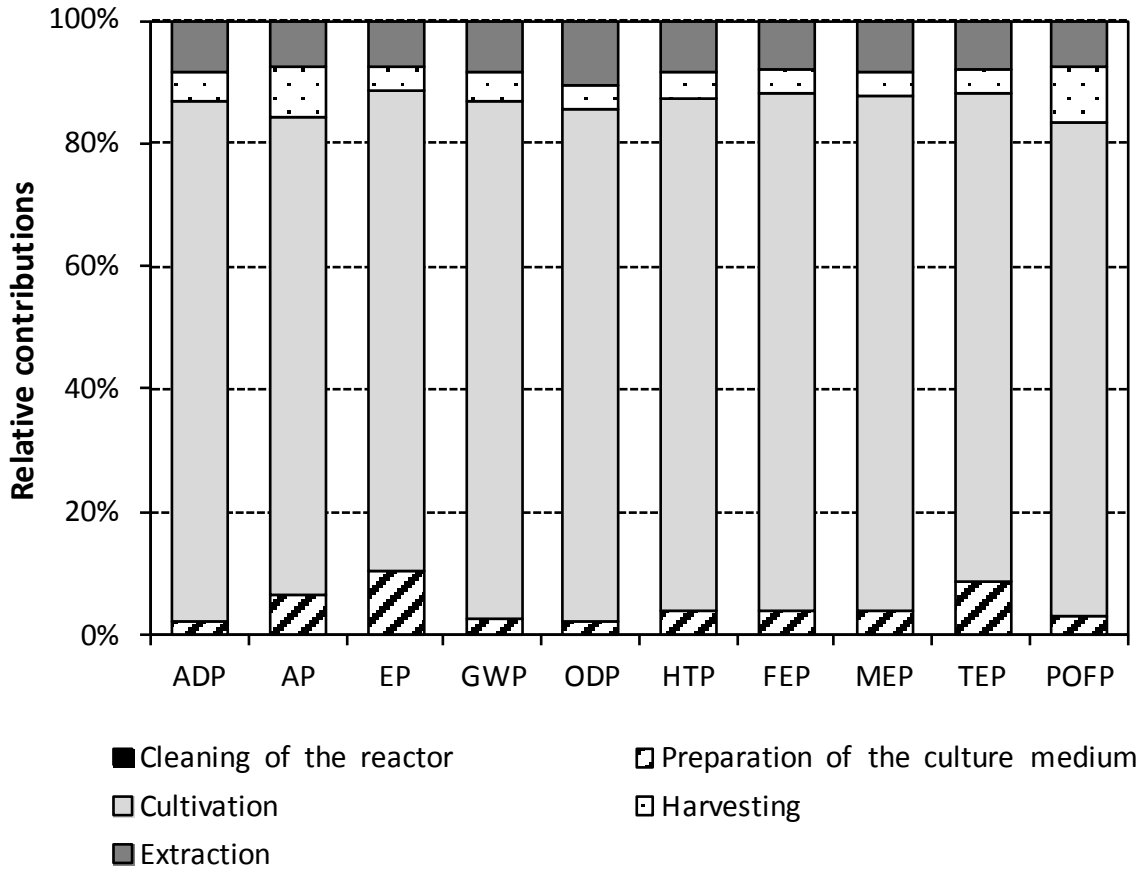


**Figure 5** Relative contribution (in %) per stage of pilot-scale process with ozone sterilisation to each impact category.

The contributing processes to each category are discussed below per impact category and stage. The breakdown of the environmental contributions is presented in **Figure 6**.

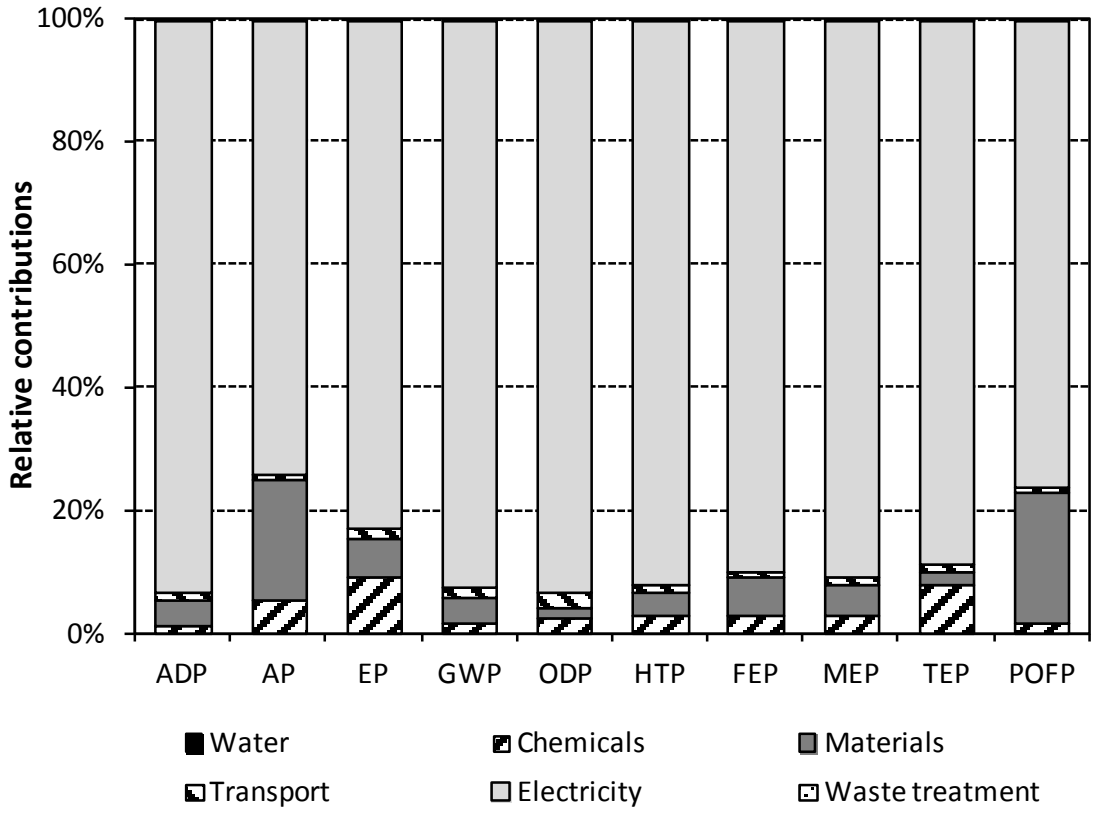


**Figure 6** Relative contribution (in %) per involved activity of pilot-scale process with ozone sterilisation to each impact category.

*Abiotic depletion potential*. The production of the electricity requirements for the pilot-scale process was responsible for 93% of contributions to ADP, especially due to the consumption of electricity in the cultivation stage. On the contrary, the production of materials for the equipment, which had a remarkable contribution for the lab-scale process, was associated to only 4% of the impact of the pilot system. This significant decrease can be probably attributed to the scale economy that promotes a more intensive use of the equipments for a longer period of time. All the other processes also had very slight contributions, being the production of the materials for the equipments the most relevant one (4%).

*Acidification potential.* The production of electricity was again the main contributor to AP (74%). $SO_2$ emissions from coal power plants were the main responsible for the impact derived from the production of electricity. In this case, the relative impact of the chemicals was noticeably reduced (from 34% to 5%) thanks to the use of supercritical $CO_2$ extraction instead of conventional solvent extraction. Regarding the production of materials, the reduction of this contribution with respect to that of the lab-scale case study was almost negligible (from 21% to 20%). Within this contribution, the use of stainless steel for the two photobioreactors used in the cultivation stage accounted for 58% of the impact.

*Eutrophication potential*. The production of electricity requirements was again the main factor for eutrophying emissions and its contribution to EP was around 83% of the total. Among the secondary processes, the production of the chemicals was the most relevant contribution (9%).

*Global warming potential*. A high percentage of GHG emissions (93%) were associated to the production of electricity requirements. The remaining processes had very limited contributions of less than 5% of the total impact each one.

*Ozone layer depletion potential.* The production of electricity was responsible of 93% of the contribution to this category, especially due to its dependence on fossil fuels. Once again, the other processes had no significant impacts, with less than 3% of the contributions. The main contributing substance was Halon 1211 (67%) emitted to air during the transport of natural gas used for energy production.

*Human toxicity.* This category was affected by the production of electricity in 92%, mainly caused by the emissions to air of arsenic (30%), polycyclic aromatic hydrocarbons (24%) and nickel (13%).

*Ecotoxicity potentials.* As in the previous categories, the production of the electricity was the major responsible of the environmental impacts to FEP (90%), MEP (91%) and TEP (89%). There was a remarkable reduction with respect to the lab-scale process in the relative contributions of waste treatment to FEP (from 28% to 0.02%) and MEP (from 24% to 0.02%). Emissions of nickel and beryllium to water were the most contributing factors to FEP and MEP, whereas TEP was principally affected by emissions of mercury (29%) and vanadium (27%) to the air derived from the use of coal for electricity generation and chromium VI (23%) to the soil from the distribution network.

*Photochemical oxidants formation potential.* The 77% of contributions to this category were due to the electricity consumption in the pilot-scale production process. The other significant contribution came from the production of materials for the equipments (21%). Emissions of $SO_2$ associated to the use of fossil fuels accounted for 76% of the impact in this category.

### 3.3 Improvement scenarios

According to the results shown in the previous section, the production of electricity required within the whole life cycle of the astaxanthin production at lab and pilot scale dominated the environmental burdens in all the impact categories. Several processes involved in the lab-scale process had significant contributions in some specific categories (e.g. materials affected considerably to ADP and POFP, waste treatment had a relevant impact in FEP and MEP). Nevertheless, the only secondary process that had relevant contributions to some categories in the case of pilot-scale system was the production of materials for the equipments, which affected to AP and POFP.

No straight-forward comparison between the results from this study with others from available literature can be made due to the lack of reports regarding the production of high-value added products from microalgae. Up to date, the related papers on microalgal LCA aim the identification of the environmental performance of biodiesel production from microalgae (Brentner et al., 2011; Lardon et al., 2009; Stephenson et al., 2010).

### *3.3.1. Sensitivity assessment of electricity requirement*

According to Stephenson et al. (2010) and Jorquera et al. (2010), the choice of cultivation system (e.g. air-lift tubular bioreactor, raceways, …) considerably influences the environmental results associated to microalgal production (specifically in terms of energy requirements and GWP) as cultivation represents the most intensive energy stage over the life cycle of biodiesel production (cradle-to-grave perspective). **Figure 7** shows a breakdown of the contribution of the electricity for all the stages of the production process that have consumption higher than 1% of the total. According to the results, the two step approach for cultivation is responsible for the highest ratios of the environmental burdens derived from the electricity production, due to the high light intensity requirements. Both stages need 82% of the total electricity consumption (20% associated to the growth stage and 62% for the stress stage), while any of the other stages contributes less than 10% to the impact.

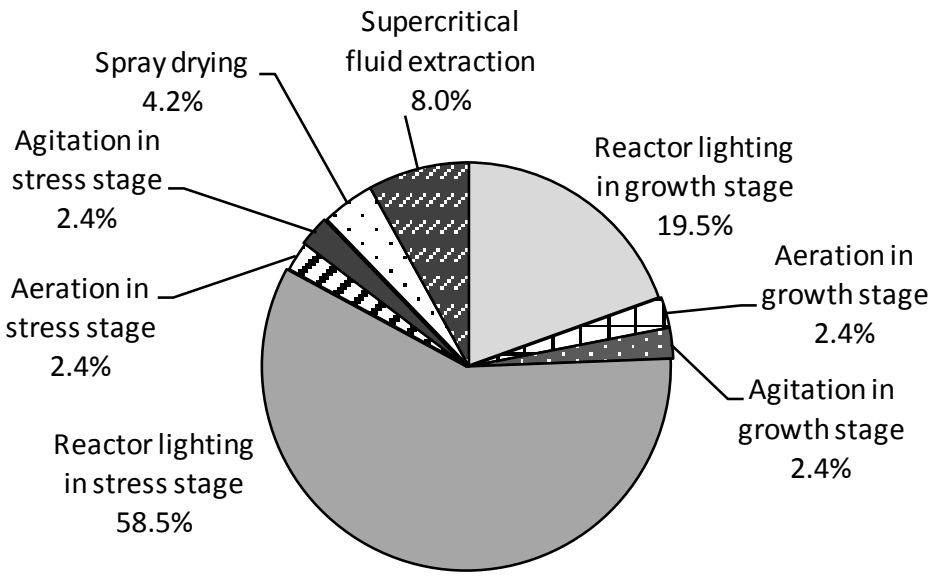


**Figure 7** Relative contributions of the electricity requirements per stage to the total environmental impact of the pilot-scale production of astaxanthin.

It could be possible to propose improvement alternatives in order to reduce the electricity requirement in the bioreactor. However, not only the electricity requirement must be taken into account in a decision process but also other important variables such as water consumption, contamination risks, light utilisation rate as well as the production yield and capacity. This is the case of the ORPs which commonly require less electricity but present lower culture productivity. Obviously, for practical, economic and environmental reasons, sunlight is preferred in extensive systems (Pruvost et al., 2011). In this pilot-scale process, two airlift PBRs were used for the experiment using artificial illumination. The use of solar illumination could be a cheap alternative although it presents limitations due to the diurnal fluctuations of light intensity that may result in a decrease in the total biomass concentration as high as 35% (Chen et al., 2011; Ogbonna et al., 1999). Specifically in Ireland, algae can only be produced outdoors for less than

five months a year and to work all the year, the installation should be moved indoors and use artificial lighting. Moreover, it is important to point out that the number of days required to obtain the same amount of microalgae cell paste under solar light conditions are considerably higher than under artificial illumination since the growth of microalgae and the composition of biomass are strongly dependent on the light supply (light source and light intensity) (Ogbonna and Tanaka, 2000; Yeh et al., 2010). For these reasons, closed controlled indoor photobioreactors illuminated with artificial light are being currently applied for high value products including astaxanthin (Lorenz and Cysewski, 2000; Patil et al., 2008).

Several related studies have analysed and compared differences on energy requirement and operational parameters between different types of PBRs under solar radiation (Brentner et al., 2011; Jorquera et al., 2010; Sierra et al., 2008). Based on these results, a sensitivity assessment was carried out considering other alternatives for the PBRs used here, which have been considered as internally-illuminated annular photobioreactors according to productivity and electricity requirements. In this study, artificial illumination is supplied to the PBR by means of fluorescent bulbs for 8 days and 16 $h \cdot day^{-1}$ for the growth stage and for other 8 days and 24 $h \cdot day^{-1}$ for the stress stage. According to Brentner et al. (2011), reductions up to 96% in the energy consumption can be achieved if flat-panel PBRs are used instead of annular PBRs when solar radiation is used as light source and considering the same amount of biomass production. Moreover, we have also considered a reduction in the biomass and astaxanthin yields taking into account the highest residence times required in a PBR under sunlight conditions in comparison with artificial light in order to obtain identical levels of biomass (Pruvost et al., 2011). Since the exposure to solar light is only diurnal (~10 hours per day), it was assumed a reduction on the biomass production for the same period of time as in annular PBRs under artificial light (8 days

for each cultivation stage) of 50% for each of the annular PBRs under sunlight. Based on Brentner et al. (2011), algal cultivation in flat-panel PBRs require the same residence time than in annular PBRs under identical conditions. Consequently, it was assumed the same production of biomass in both annular and flat-panel PBRs.

Therefore, in the sensitivity assessment, annular and flat-panel PBRs with sunlight have been proposed as potential alternatives to the artificially illuminated PBRs, assuming no differences in other LCI data (Brentner et al. 2011). The scenarios proposed should be the following:

- Sc 1: annular PBRs with artificial light in growth and stress stages and 800 g of astaxanthin production
- Sc 2: annular PBRs with sunlight in growth and stress stage and 400 g of astaxanthin production
- Sc 3: flat-panel PBRs with artificial light in growth and stress stages and 800 g of astaxanthin production
- Sc 4: flat-panel PBRs with sunlight in growth and stress stages and 400 g of astaxanthin production

Although longer lifetimes for the reactor materials are expected for the flat-panel PBRs (50 years in comparison with 40 years considered for the annular PBRs) as well as larger required areas for similar biomass yield, these values have not been taken into account due to their minimal contribution to most of the impact categories. **Figure 8** shows the comparative environmental result per impact category and PBR models assumed. According to the results, all the proposed scenarios would allow significant environmental benefits with respect to the case study. In the first alternative scenario, in which the use of annular PBRs was considered and artificial illumination was substituted by sunlight illumination, the obtained improvements were limited

between 15% and 46% (for AP and GWP respectively). For the flat-panel configuration with sunlight illumination, reductions of impact ranged from 29% to 64%. The ideal situation would be the use of two flat-panel PBRs with artificial illumination, which would permit a decrease between 62% and 79% of the impacts in all categories.

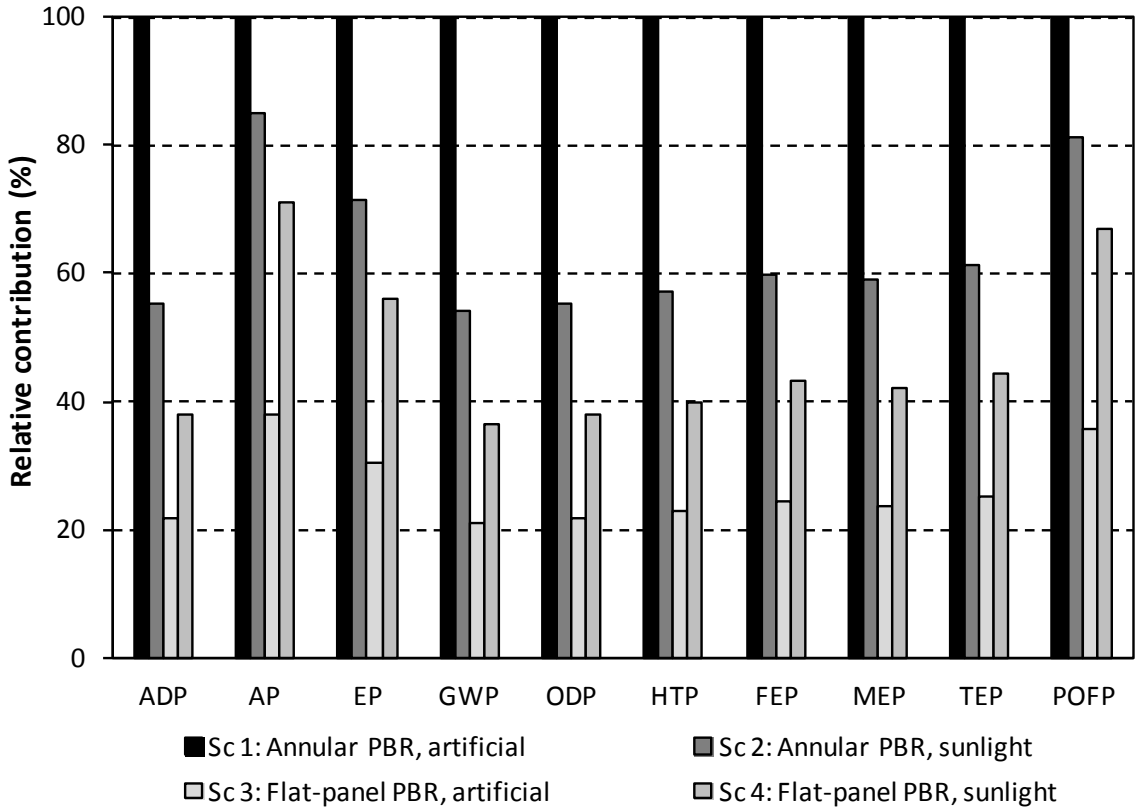


**Figure 8** Sensitivity analysis of the environmental performance considering four different configurations for the two PBRs: annular and flat-panel PBRs with artificial or solar illumination.

*3.3.2. Exploitation of co-products from residual algal paste*

Due to the potential uses of astaxanthin, this paper was focused on its natural production (at lab and pilot-scale) by the green microalgae *Haematococcus pluvialis*. Although the main objective

in the process is the production of this carotenoid, a co-product from the extraction stage known as “algal paste” also has potential applications for biodiesel production due to its fatty acids content. Alternatively it could be feedstock for an anaerobic digester or used as a fertiliser (Campbell et al., 2011). Although it was not taken into account within the system boundaries, further research should be paid on the algal by-products.

## 4 Conclusions

Nowadays special interest is being paid to microalgal production for several reasons: sustainable energy, foodstuffs, industrial chemicals, pharmaceuticals and/or nutraceuticals production. The life cycle impacts of microalgae cultivation considerably depend on the production scale, according to the results presented in this study. Moreover, several remarkable improvements observed in the pilot process can be related to changes implemented in the system as a result of lab-scale environmental assessment, such as the substitution of compressed air supply or the use of supercritical $CO_2$ extraction as a more suitable separation technique. In the lab-scale process, several inputs affected the environmental profile, whereas in the case of pilot system, electricity dominated the contributions to all categories.

The choice of the photobioreactor considered for the algae cultivation stage is one of the most important environmental issues to be taken into account due to the large differences of electricity requirements. Moreover, if the microalgae are cultivated in order to obtain a specific compound such as carotenoids, the extraction method considered, whether it is based on organic solvents or supercritical fluids, has significant influence on the environmental results. Due to the high contribution of the electricity, a sensitivity assessment was proposed in order to identify the best reactor configuration for the *Haematococcus* astaxanthin production system from an

environmental point of view. According to the results, the scenarios based on the use of sunlight instead of artificial illumination allowed significant reductions of impact. However, the improvements observed in these cases were limited by the decrease in biomass productivity associated with sunlight culture systems. Therefore, the optimal production system would consist of two flat-panel PBRs with artificial illumination, which would allow reductions between 62% and 79% of the impact depending on the considered category. As this study included the evaluation of a pilot-scale process, the results allow the identification of specific environmental hot spots which are likely to affect the industrial scale processes and, thus, that must be solved before the implementation of the commercial process. Technological advantages are rapidly occurring in the microalgae related industries. The results of this paper should be considered in order to produce in a more sustainable manner not only *Haematococcus* astaxanthin but also other microalgae derived compounds.

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**Acronyms**

ADP = Abiotic depletion

AP = Acidification

DMSO = Dimethyl sulfoxide

EP = Eutrophication

FEP = Freshwater aquatic ecotoxicity

GHG = Greenhouse gas

GWP = Global warming

HTP = Human toxicity

LCA = Life Cycle Assessment

LCI = Life Cycle Inventory

MEP = Marine aquatic ecotoxicity

ODP = Ozone layer depletion

ORP = Open raceway ponds

PBR = Photobioreactor

PVC = Polyvinyl chloride

POFP = Photochemical oxidants formation

TEP = Terrestrial ecotoxicity

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