Environmental life cycle optimization of essential terpene oils produced by the macroalga *Ochtodes secundiramea*

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Abstract

The macroalga *Ochtodes secundiramea* is a well-known producer of essential terpene oils with promising biological activities and similar applications to those of microalgal biocompounds in the pharmaceutical, food or cosmetics sectors. This study assesses the environmental impacts associated with the production of five essential terpene oils (myrcene, 10Z-bromomyrcene, 10E-bromo-3-chloromyrcene, apakaochtodene B and acyclic $C_{10}H_{14}Br_2$) by *O. secundiramea* cultivated in a closed airlift photobioreactor with artificial illumination. The results of the life cycle assessment (LCA) allowed analyzing the effect of implementing a semi-continuous operation on several stages of the life cycle of the products, which may lead to impact reductions from 1% up to 25%.

Regarding the most problematic aspects of the process, the cultivation in the photobioreactor (S4) was identified as the main stage responsible for the environmental burdens, with contributions ranging between 60% and 80% of the total impacts for a semi-continuous production maintained during one year of operation. The production of electricity is the key activity affecting eight of the ten assessed categories and involves between 50% and 60% of the impact of the process. S4 is the main cause of the high energy requirements, with 86% of the total electricity consumption. Additionally, several scenarios aiming at improving the environmental profile of the system were evaluated. The application of LCA finally led to the proposal of two optimized scenarios with improvements between 8% and 40% with respect to the baseline case study.

Keywords Essential terpene oils, *Ochtodes secundiramea*, Life Cycle Assessment, macroalgae, photobioreactor, environmentally optimized scenarios

1. Introduction

Macrophytic marine algae (known as macroalgae or seaweeds) have been extensively cultured and collected from natural aquatic habitats, especially in Asian countries, as a source of food and chemicals (Aresta et al., 2005; Rorrer and Cheney, 2004). Their utilization worldwide involves a multi-billion dollar industry, mainly related to the production of agar, carrageenan and alginate. Although most of the commercial exploitation is linked to hydrocolloids used in various sectors for their gelling, water-retentive and emulsifying properties, new applications are receiving increasing attention (Smit, 2004).

These uses include the production of supplements for functional foods or cosmetics and macroalgae are currently being investigated for pharmaceutical purposes (Andrade et al., 2013; Balboa et al., 2015; Holdt and Kraan, 2011; Smit, 2004). This is supported by their richness in polysaccharides, minerals and certain vitamins, as well as other bioactive substances such as proteins, lipids and polyphenols with properties as antibacterial, antifungal, anti-inflammatory, antioxidant, antitumor and antiviral agents, among others (Andrade et al., 2013; Jung et al., 2013; Smit, 2004).

As in the case of microalgae, seaweeds have superior photon conversion efficiency and can synthesize biomass from sunlight, CO_2 and inorganic nutrients faster than terrestrial plants. They show high production yields per unit area and high rates of CO_2 fixation. In addition, their depolymerization is easier since they lack hemicellulose and lignin, which is present in terrestrial plants for structural support (Aresta et al., 2005; Wei et al., 2013).

There are two general approaches for macroalgal cultivation. One option corresponds to the vegetative cultivation (used in this case study for a single strain), in which small fragments of the cultured species are grown in aquatic systems under appropriate conditions (e.g. temperature, light, salinity, nutrients) and later harvested (Fasahati et al., 2015; McHugh, 2003; Wei et al., 2013). Some species (e.g. *Laminaria* sp.) can only be grown through a reproductive cycle. In this second approach, the protocol requires transitions from spores released by grown seaweed (sporophytes) to gametophytes, which join to form embryonic sporophytes that can finally grow in the aforementioned vegetative systems and be harvested (McHugh, 2003). The transitions can take place in nursery tanks or ponds and in controlled photobioreactors (McHugh, 2003; Rorrer and Cheney, 2004).

The most common setups for macroalgal cultivation are sea-based farming sites that include offshore and near-shore coastal farms, together with land-based ponds (McHugh, 2003; Wei et al., 2013). Although offshore farming has been successfully tested, its cost is still high. Near-shore farms are common in some countries (e.g. China and Japan), but pose some environmental concerns for which government regulations in other areas have prevented their use. Land-based pond systems present several advantages including the easy control of nutrients and conditions, as well as the possibility to integrate their production with other aquaculture species, while avoiding adverse weather, disease and predation. However, the use of these systems is restricted to the optimization of technology and the construction costs in scaled-up systems (Wei et al., 2013). Moreover, these techniques may not be suitable for the production of high value metabolites, due to contamination risks and absence of controlled conditions.

Despite the great potential of macroalgae as sources of numerous bioactive metabolites, the lack of appropriate *in vitro* culture systems for continuous supply is a major barrier for bioprocess development (Rorrer and Cheney, 2004). With this regard, several artificial systems, such as ponds and enclosed photobioreactors (PBRs), are currently proposed as a suitable alternative to *in situ* conventional farming sites for the commercial implementation of seaweed cultivation (Ahmed and Taha, 2011; Rorrer and Cheney, 2004). As in the case of microalgae, the use of artificial systems allows the growth of target species on low-value land (Ahmed and Taha, 2011), while avoiding possible impacts of large-scale seaweed farming on biodiversity (Radulovich et al., 2015; Wei et al., 2013).

Techniques from tissue culture engineering can be exploited to produce asexual, omnipotent, regenerated plantlet tissues from macroalgal callus cultures (Maliakal et al., 2001). These plantlet tissues are highly amenable to cultivation within photobioreactors

(Barahona and Rorrer, 2003). Cell and tissue cultivation in PBRs enable a continuous and steady production of macroalgal products with high yields and no seasonality barriers (Ahmed and Taha, 2011). Moreover, PBRs provide controlled conditions under sterile environment, which are required for the production of high value metabolites (Rorrer and Cheney, 2004). The use of these techniques relies on the development of three components: i) cell and tissue culture, ii) PBR design and iii) identification of strategies to favor the production of target metabolites (Ahmed and Taha, 2011; Rorrer and Cheney, 2004).

Despite being still considerably underdeveloped, the techniques for cell and tissue culture are mainly based on those for land plants. Their characteristics depend on the species and its morphology. Thus, the development of cell culture systems for filamentous algae is based on diverse isolation techniques, whereas tissue culture systems usually involve a first step of callus induction from explants of specimens collected on site and a second step of partial regeneration of shoot tissues to form "microplantlets" (Rorrer and Cheney, 2004).

Once developed, the cell or tissue culture is suspended in the PBR. The cultivation requires illumination, nutrient delivery and appropriate gas exchange (CO_2 addition and O_2 removal), as well as mixing and temperature control. The main bioreactor configurations reported for macroalgal cultures are airlift PBRs, bubble columns, stirred tanks and tubular recycled PBRs (Rorrer and Cheney, 2004; Yong et al., 2014). Each of them presents advantages and limitations regarding mixing patterns, gas transfer, light efficiency and shear damage potential, so the selection depends on the species and required conditions (Rorrer and Cheney, 2004).

Future research related to the production of metabolites from macroalgae should focus on the development of the third component: the design of appropriate strategies to

enhance the production of target compounds. With this purpose, some authors have investigated the application of metabolic principles for the regulation of secondary metabolism of some algal species (Maliakal et al., 2001; Polzin et al., 2003; Wargacki et al., 2012).

After cultivation, algae can be harvested by conventional manual methods or recently developed harvesting systems for large quantities (Bruton et al., 2009; Wei et al., 2013). Finally, suitable extraction techniques need to be selected for the efficient separation and purification of target compounds, including conventional techniques based on heat or solvent use and novel options such as supercritical fluid extraction or pressurized liquid extraction (Kadam et al., 2013).

In this study, a novel system for the controlled cultivation of *Ochtodes secundiramea* (Montagne) MA Howe is proposed. *O. secundiramea* is a red macroalga that exhibits a significant content of diverse bioactive compounds belonging to the group of essential terpenes (Machado et al., 2014). Terpenes constitute a vast group of natural products, based on the different possible arrangements of bonded isoprene units (C_5H_8) (Zwenger and Basu, 2008). The existing arrangements extend from single isoprene units, known as hemiterpenes, to combinations of eight (tetraterpenes) or even more units (polyterpenes), monoterpenes (containing two isoprene units) being the most abundant type, followed by sesquiterpenes (consisting of three isoprene units) (Silvestre and Gandini, 2008; Zwenger and Basu, 2008).

Essential terpene oils are secondary metabolites commonly obtained from herbs and other plants (e.g. rosemary, juniper, pine, eucalyptus), although they are also found in some insects, marine organisms and fungi (Mühlbauer et al., 2003; Silvestre and Gandini, 2008). Among marine sources, macroalgae are increasingly proposed as an alternative source of these essential oils (Barahona and Rorrer, 2003; Mühlbauer et al.,

2003). In nature, many terpenes play important ecological roles, such as their defense function as insect repellents or their involvement in symbiotic mechanisms. Moreover, they can be used for a wide variety of applications such as the production of insecticides and polymers, as well as for cosmetics and pharmaceutical products (Silvestre and Gandini, 2008).

In particular, *O. secundiramea* contains considerable amounts of halogenated monoterpenes. A variety of assays performed on *O. secundiramea* extracts have allowed the identification of powerful antifungal activity linked to these terpenes (Machado et al., 2014). Furthermore, several of these compounds have been found to possess anticancer and anti-microbial bioactivities (Polzin, 2005).

Due to the potential interest of *O. secundiramea* cultivation, a semi-continuous production process is here proposed and the subsequent extraction of essential terpene oils, specifically myrcene, 10Z-bromomyrcene, 10E-bromo-3-chloromyrcene, apakaochtodene B and acyclic $C_{10}H_{14}Br_2$. LCA methodology was selected as a suitable environmental management tool for the evaluation of the impacts of the process throughout its whole life cycle.

2. Goal and scope definition

The aim of this LCA study is to identify the environmental impacts associated with the complete cultivation and extraction process according to a cradle-to-gate perspective. In a first step, the impacts are quantified with respect to the total terpene essential oils. To do so, the selected functional unit (FU) is 700 mg of essential terpene oils (one batch of production). Subsequently, the specific impacts of the different fractions are estimated. Since the five bioactive compounds have similar applications and economic value, a mass allocation will be considered to assign the corresponding impacts to each product.

Additionally, the different stages and activities involved in the process are analyzed to identify the highest contributions to the environmental profile and propose alternatives.

The production scheme considered in this assessment is based on the lab process developed by the Bioengineering Group of the Earth and Life Institute at the University of Louvain (Belgium). The cultivation was carried out in a 13 L transparent polyvinyl chloride (PVC) airlift PBR. The process was divided into the following six main stages: cleaning and sterilization (S1), preparation of the culture medium (S2), preparation of the inoculum (S3), cultivation in PBR (S4), cell separation (S5) and cell disruption and extraction (S6). **Figure 1** shows the different stages and processes included within the system boundaries.

- S1. Cleaning and sterilization: Before each batch culture in the 13 L PBR, a cleaning stage was carried out. Firstly, 20 L of tap water with about 10 mL soap, followed by 5 L of sterile demineralized water, were used to clean the reactor. Subsequently, the PBR was loaded with sterilized culture medium and sparged with 2.0 L $O_2 \cdot min^{-1}$ (source pressurized oxygen tank, reduced to 1 bar before injection) passed through an O_3 generator to further disinfect the inside of the PBR. The reactor was sparged for one additional day with an air flow of 2.0 $L \cdot min^{-1}$ (compressed house air at 6 bar, reduced to 1 bar before injection) to remove all traces of O_3 before inoculation.
- S2. Preparation of the culture medium: A total volume of 20 L of sterile seawater was prepared to be used in all steps of the process. The artificial seawater medium (ASM), based on the concentrations reported by Jeffryes et al. (2008), was composed of 7.64 mM KCl, 1.97 mM $NaHCO_3$, 690 µM KBr, 354 µM H_3BO_3, 63.3 µM NaF, 345 mM NaCl, 23.8 mM Na_2SO_4, 44.9 mM $MgCl_2 \cdot 6H_2O$, 8.7 mM $CaCl_2 \cdot 2H_2O$ and 77.7 µM $SrCl_2 \cdot 6H_2O$. The composition

of micronutrients in the medium was 3 µM KI, 19.7 nM $FeCl_3$, 0.21 µM $C_{10}H_{14}O_8N_2Na_2{\cdot}2H_2O$ (EDTA), 0.23 µM $MnSO_4{\cdot}4H_2O$, 16.5 nM $ZnSO_4{\cdot}7H_2O$, 8 nM NH_4VO_3, 3.59 nM $Na_2MoO_4{\cdot}2H_2O$, 4.54 nM $CuSO_4{\cdot}5H_2O$, 0.10 nM Na_2SeO_3, 2.47 nM $CoSO_4{\cdot}5H_2O$ and 0.6 nM $NiCl_2{\cdot}6H_2O$. The composition of macronutrients supplement was 10.6 mM $NaNO_3$ and 483 µM $NaH_2PO_4{\cdot}H_2O$. The ASM also contained 1.53 nM thiamine HCl, 0.737 pM biotin and 5.71 pM vitamin B12. The salts used in these preparations were reagent grade chemicals and demineralized water was used to prepare all medium components. The medium was autoclaved for 30 min at 121°C and 1.5 bar.

- S3. Preparation of the inoculum: Firstly, 400 mL cell cultures were kept in 500 mL culture bottles within an incubator set at 25°C and 60 µmol photons$\cdot m^{-2}\cdot s^{-1}$ light intensity from two 18 W fluorescent lights in a 14 h on, 10 h off diurnal cycle. Each bottle was aerated with 300 mL$\cdot min^{-1}$ of sterile air (0.75 vvm). The medium was changed once per week in each culture bottle when the plantlets were separated from the spent medium by coarse filtration, rinsed with 200 mL of water demineralized by ion exchange, and returned to the culture bottle with 400 mL of medium previously sterilized in autoclave. The cultures were inoculated at 10 g$\cdot L^{-1}$ fresh weight (2 g$\cdot L^{-1}$ dry weight) and grown to 20 g$\cdot L^{-1}$ fresh weight (4 g$\cdot L^{-1}$ dry weight) after two weeks.

  After one cell subculture cycle, 32 g of fresh plantlets from the 500 mL culture bottles was used to inoculate a 5 L Scott flask containing 4 L of fresh medium, aerated with 3 L$\cdot min^{-1}$ of sterile filtered house air and illuminated by two 18 W fluorescent bulbs. At days 7 and 14, 80 mL of macronutrient solution was added to the culture medium. After 21 days, the 5 L culture flask contained 65 g of fresh plantlets, which were used to inoculate the 13 L PBR.

- S4. Cultivation in PBR: The airlift PBR was loaded with 13 L of sterilized culture medium and inoculated with 65 g fresh plantlets (13 g dry mass) which yielded an initial cell culture density of 1 $g_{DW} \cdot L^{-1}$. The reactor was illuminated by six fluorescent bulbs of 36 W each and aerated by 2.6 $L \cdot min^{-1}$ of compressed air. The pH was maintained at 8.0 with pure CO_2 at 1.5 bar. Artificial seawater medium and macronutrient solution was fed into the PBR at a rate of 464 and 42 $mL \cdot d^{-1}$, respectively, by the use of an intermittent timer coupled to a peristaltic pump, operated for 15 min every 4 h. The culture was kept for 28 days, yielding a final biomass of 780 g fresh macroalgae (equivalent to 156 g_{DW}).
- S5. Cell separation: In this step, 13 L of cell culture (780 g of fresh plantlet biomass) was transferred by gravity into a carboy. After 1 min, the plantlets settled to the bottom and approximately 10 L of supernatant were removed. Successive aliquots of the remaining tissues were passed onto a large strainer to remove the liquid. Fresh tissues (65 g) were kept to inoculate the following photobioreactor cultivation, while the remaining 715 g of fresh plantlets (containing 700 mg of essential terpene oils) were washed first with 13 L of tap water followed by 13 L of deionized water. The plantlets were placed in a filter (cotton cloth) and pressed to absorb the remaining moisture. The cloth was dried at room temperature.
- S6. Cell disruption and extraction: 715 g of fresh microplantlets were grinded using a mortar and pestle and 4 L of liquid nitrogen to maintain the tissues in a frozen state during the grinding process, until the liquid nitrogen was evaporated (approx. 3 min). The biomass was then placed in a screw-cap flask with 1000 mL of dichloromethane (DCM) for 24 h at 22°C under continuous mixing on an orbital shaker at 100 rpm. After collecting the extraction solvent, the solid

residue was vacuum filtered and the biomass was re-extracted using the same conditions. The combined extract was evaporated under nitrogen gas flow at 22°C to give a total amount of 700 mg of essential oils.

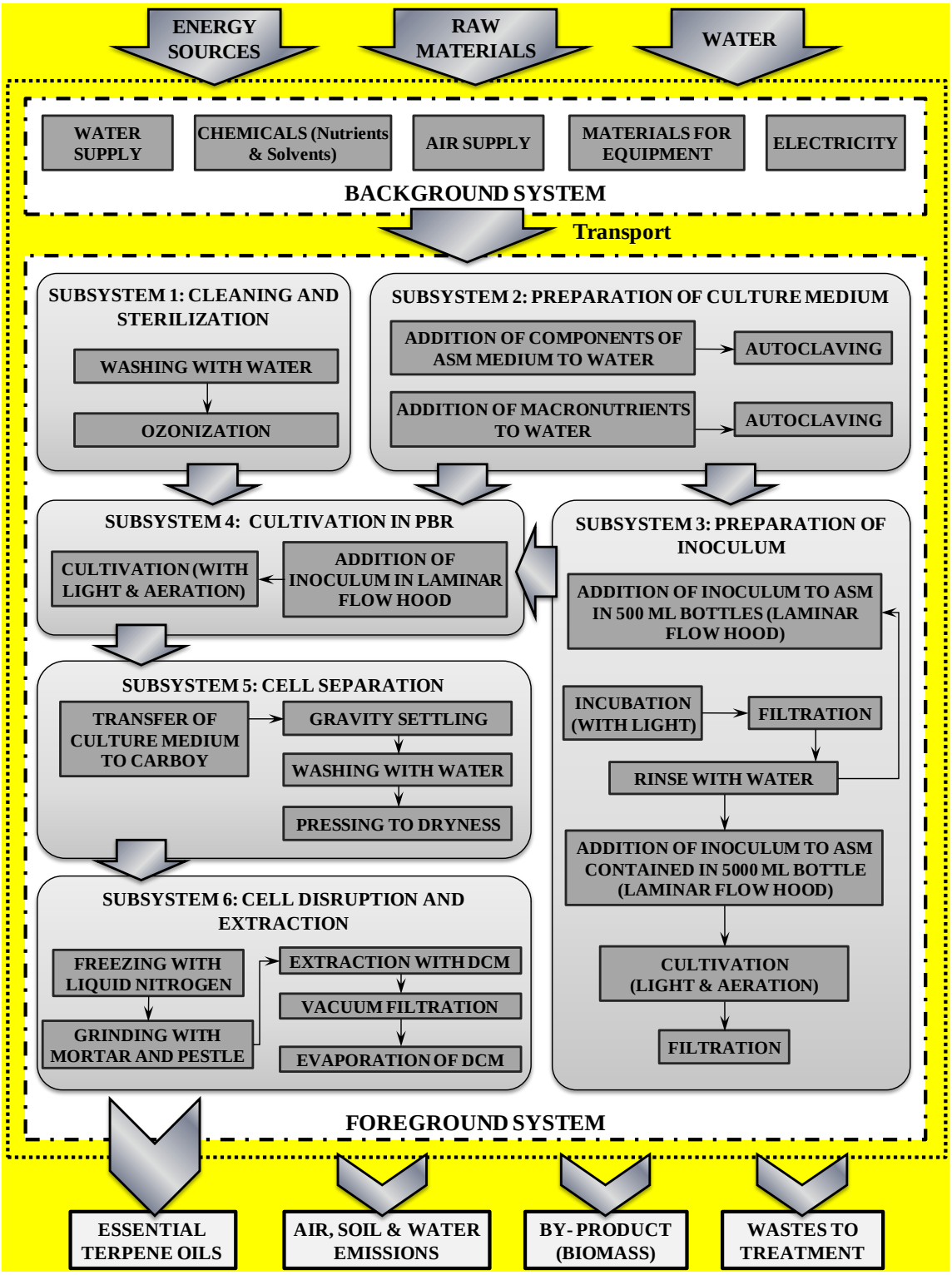

Figure 1. Process chain and system boundaries of the cultivation of *Ochtodes secundiramea* in an airlift PBR and subsequent extraction of essential terpene oils.

Since the required amount of fresh tissue to inoculate the reactor can be supplied by taking a small portion of the biomass obtained in S5, the subculture in intermediate volumes (500 mL and 5 L flasks) would be unnecessary when implementing a semi-continuous process on subsequent batches. For this reason, three possible scenarios are proposed and compared:

i) Discontinuous operation, corresponding to one batch that includes the six described stages.

ii) Semi-continuous operation for approximately 1 year (12 batches) with stages S3 and S4 only performed for the first batch, whereas the subsequent cultivations in the 13 L PBR are started by re-inoculating a portion of the harvested biomass.

iii) Semi-continuous operation maintained for 10 years (120 batches) with each inoculum coming from a portion of the harvested biomass.

3. Life cycle inventory, data quality and assumptions

The life cycle inventory (LCI) data for the foreground system (i.e. water, chemicals and electricity consumptions as well as transport distances) consisted of average data from on-site measurements. Regarding water emissions derived from the different production stages, they were assumed to be directly discharged to the environment. The global inventory of the process is shown in **Table 1** for the three possible scenarios: 1 batch, 1 year of semi-continuous operation (35 days for inoculum preparation followed by 12 batches) and 10 years of semi-continuous operation (35 days for inoculum preparation followed by 120 batches).

Table 1. Inventory table for the production of essential terpene oils by *O. secundiramea* cultivated in an airlift PBR (FU=700 mg terpenes, equivalent to 1 batch)

INPUTS from TECHNOSPHERE			
	One batch	1-year semi-continuous mode	10 years semi-continuous mode
Materials			
S1. Cleaning and sterilization			
Tap water	20.00 L	20.00 L	20.00 L
Sterile water	5.00 L	5.00 L	5.00 L
Soap	10.00 mL	10.00 mL	10.00 mL
Stainless steel	32.43 g	32.43 g	32.43 g
Compressed air			
S2. Preparation of culture medium			
Demineralized water	34.53 L	27.78 L	27.23 L
KCl	18.91 g	15.14 g	14.84 g
$NaHCO_3$	5.43 g	4.40 g	4.31 g
KBr	2.73 g	2.18 g	2.14 g
H_3BO_3	0.73 g	0.58 g	0.57 g
NaF	0.09 g	0.07 g	0.07 g
NaCl	669.24 g	536.16	525.28 g
Na_2SO_4	112.21 g	89.90 g	8.07 g
$MgCl_2{\cdot}6H_2O$	302.99 g	242.74 g	237.81 g
$CaCl_2{\cdot}2H_2O$	42.45 g	34.01 g	33.32 g
$SrCl_2{\cdot}6H_2O$	0.69 g	0.55 g	0.54 g
KI	16.53 mg	13.24 mg	12.97 mg
$FeCl_3$	1.06 mg	0.85 mg	0.83 mg
EDTA	2.56 mg	2.05 mg	2.01 mg
$MnSO_4{\cdot}4H_2O$	1.69 mg	1.35 mg	1.32 mg
$ZnSO_4{\cdot}7H_2O$	0.16 mg	0.13 mg	0.12 mg
NH_4VO_3	31.06 µg	24.89 µg	24.38 µg
$Na_2MoO_4{\cdot}2H_2O$	28.83 µg	23.10 µg	22.63 mg
$CuSO_4{\cdot}5H_2O$	37.63 µg	30.14 µg	29.53 mg
$CoSO_4.5H_2O$	20.09 µg	16.10 µg	15.77 mg
$NiCl_2{\cdot}6H_2O$	4.73 µg	3.79 µg	3.72 µg
Na_2SeO_3	0.55 µg	0.44 µg	0.43 µg
Thiamine HCl	17.13 µg	13.72 µg	13.44 µg
Biotin	5.97 ng	4.79 ng	4.69 ng
Vitamin B12	0.26 µg	0.21 µg	0.20 µg
$NaNO_3$	1.20 g	1.07 g	1.06 g
$NaH_2PO_4{\cdot}H_2O$	89.04 mg	79.27 mg	78.47 mg
Stainless steel	5.49 g	4.42 g	4.33 g
S3. Preparation of inoculum			
Stainless steel	2.83 g	0.24 g	0.02 g
Glass	36.22 g	3.02 g	0.30 g
High density polyethylene (HDPE)	0.51 g	0.04 g	0.04 g
Lamps	3.32 g	0.28	0.03 g
Polyurethane foam	43.78 g	3.65 g	0.36 g
Zinc coated steel	102.17 g	8.51 g	0.85 g
Silicon rubber	1.67 g	0.14 g	0.01 g
Demineralized water	20.00 mL	16.67 mL	1.67 mL
Compressed air (8 bar)	12.85 m^3	1.07 m^3	0.11 m^3
S4. Cultivation in PBR			
Stainless steel	30.74 g	30.37 g	27.98 g
PVC	32.59 g	32.17 g	29.43 g
Lamps	34.27 g	34.27 g	34.27 g
Compressed air (8 bar)	13.10 m^3	13.10 m^3	13.10 m^3

Table 1. Inventory table for the production of essential terpene oils by *O. secundiramea* cultivated in an airlift PBR (FU=700 mg terpenes, equivalent to 1 batch) (*Cont.*)

INPUTS from TECHNOSPHERE			
	One batch	One year semi-continuous mode	10 years semi-continuous mode
Materials			
S5. Cell separation			
Polypropylene (PP)	16.52 g	16.30 g	14.92 g
Filter (cloth)	7.59 g	7.49 g	6.86 g
Tap water	13.00 L	13.00 L	13.00 L
Deionized water	13.00 L	13.00 L	13.00 L
S6. Cell disruption and extraction			
Liquid nitrogen	4.00 L	4.00 L	4.00 L
Gaseous nitrogen	1000 L	1000 L	1000 L
Dichloromethane	2.00 L	2.00 L	2.00 L
Steel	127.35 g	127.35 g	127.35 g
Glass	18.18 g	18.18 g	18.18 g
Energy			
S1. Cleaning and sterilization			
Autoclaving	0.19 kWh	0.19 kWh	0.19 kWh
Ozone generator	1.92 kWh	1.92 kWh	1.92 kWh
Air supply	0.05 kWh	0.05 kWh	0.05 kWh
S2. Preparation of culture medium			
Autoclaving	1.29 kWh	1.05 kWh	1.03 kWh
S3. Preparation of inoculum			
Laminar flow hood	0.93 kWh	0.08 kWh	0.01 kWh
Incubation (excluding lights)	4.48 kWh	0.37 kWh	0.04 kWh
Aeration	1.68 kWh	0.14 kWh	0.01 kWh
Lighting	12.47 kWh	1.04 kWh	0.09 kWh
S4. Cultivation in PBR			
Laminar flow hood	0.75 kWh	0.75 kWh	0.75 kWh
Aeration	1.72 kWh	1.72 kWh	1.72 kWh
Lighting	84.67 kWh	84.67 kWh	84.67 kWh
Medium pumping	2.10 kWh	2.10 kWh	2.10 kWh
S6. Cell disruption and extraction			
Extraction with DCM on orbital shaker	9.60 kWh	9.60 kWh	9.60 kWh
Vacuum filtration	0.13 kWh	0.13 kWh	0.13 kWh
Solvent evaporation	0.56 kWh	0.56 kWh	0.56 kWh
Transport			
Truck 3.5-7.5, euro 4:			
S1. Cleaning and sterilization			
Equipment	19.46 kg·km	19.21 kg·km	17.62 kg·km
Wastes	1.62 kg·km	1.60 kg·km	1.47 kg·km
S2. Preparation of culture medium			
Chemicals (nutrients)	925.89 kg·km	741.87 kg·km	726.82 kg·km
Materials (equipment)	3.30 kg·km	2.65 kg·km	2.60 kg·km
Wastes	0.27 kg·km	0.22 kg·km	0.22 kg·km
S3. Preparation of inoculum			
Materials (equipment)	114.29 kg·km	9.52 kg·km	0.95 kg·km
Wastes	9.52 kg·km	0.79 kg·km	0.08 kg·km
S4. Cultivation in PBR			
Materials (equipment)	58.56 kg·km	58.08 kg·km	55.01 kg·km
Wastes	4.88 kg·km	4.84 kg·km	4.58 kg·km

Table 1. Inventory table for the production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR (FU=700 mg terpenes, equivalent to 1 batch) (*Cont.*)

	One batch	1-year semi-continuous mode	10 years semi-continuous mode
INPUTS from TECHNOSPHERE			
Transport			
S5. Cell separation			
Chemicals (solvents)	2.13 tkm	2.13 tkm	2.13
Materials (equipment)	87.32 kg·km	87.32 kg·km	87.32 kg·km
Wastes	7.28 kg·km	7.28 kg·km	7.28 kg·km
INPUTS from ENVIRONMENT			
S3. Preparation of inoculum			
Algal biomass for inoculum	16.00 g	1.33 g	0.13 g
Carbon dioxide, CO_2([1])	13.86 g	1.16 g	0.11 g
S4. Cultivation			
CO_2([1])	202.31 g	202.31 g	202.31 g
OUTPUTS to TECHNOSPHERE			
Product			
Essential terpene oils	700 mg	700 mg	700 mg
By-product			
Cell paste, recycled to PBR	0	59.58 g	64.46 g
Residual cell paste	779.30 g	719.72 g	714.84 g
Wastes to landfill			
S1. Cleaning and sterilization			
Steel	32.43 g	32.02 g	29.37 g
S2. Preparation of culture medium			
Steel	5.49 g	4.42 g	4.33 g
S3. Preparation of inoculum			
Steel	104.99 g	8.75 g	0.87 g
Glass	36.22 g	3.02 g	0.30 g
HDPE	0.51 g	0.04 g	0.04 g
Polyurethane foam	43.78 g	3.65 g	0.36 g
Silicon rubber	1.67 g	0.14 g	0.01 g
S4. Cultivation in PBR			
Steel	30.74 g	30.36 g	27.98 g
PVC	32.59 g	32.17 g	29.43 g
S5. Cell separation			
PP	16.51 g	16.30 g	14.92 g
Filter (cloth)	7.59 g	7.50 g	6.86 g
S6. Cell disruption and extraction			
Steel	127.35 g	127.35 g	127.35 g
Glass	18.18 g	18.18 g	18.18 g
Wastes to specific treatment			
S3. Preparation of inoculum			
Lamps	3.32 g	0.28	0.03 g
S4. Cultivation in PBR			
Lamps	34.27 g	34.27 g	34.27 g

[1]Corresponding to an approximate biomass composition of $CH_{2.11}O_{1.01}N_{0.055}P_{0.002}$.

Table 1. Inventory table for the production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR (FU=700 mg terpenes, equivalent to 1 batch) (*Cont.*)

OUTPUTS to ENVIRONMENT			
	One batch	1-year semi-continuous mode	10 years semi-continuous mode
Water emissions			
Wastewater (from all stages)	84.95 L	78.79	78.24 L
DCM (from S6)	2 L	2 L	2 L
Total non-consumed nutrients (from S3+S4+S5)			
KCl	0.85 g	0.74 g	0.74 g
$NaHCO_3$	0.25 g	0.22 g	0.22 g
KBr	0.12 g	0.11 g	0.11 g
H_3BO_3	0.03 g	0.03 g	0.03 g
NaF	39.86 mg	34.98 mg	34.59 mg
NaCl	30.24 g	26.54 g	26.24 g
Na_2SO_4	5.07 g	4.45 g	4.40 g
$MgCl_2 \cdot 6H_2O$	13.69 g	12.02 g	11.88 g
$CaCl_2 \cdot 2H_2O$	1.92 g	1.68 g	1.66 g
$SrCl_2 \cdot 6H_2O$	31.07 mg	27.27 mg	26.96 mg
KI	0.75 mg	0.65 mg	0.65 mg
$FeCl_3$	47.92 µg	42.06 µg	41.58 µg
EDTA	0.12 mg	0.10 mg	0.10 mg
$MnSO_4 \cdot 4H_2O$	76.27 µg	66.94 µg	66.18 mg
$ZnSO_4 \cdot 7H_2O$	7.12 µg	6.25 µg	6.17 µg
NH_4VO_3	1.49 µg	1.22 µg	1.22 µg
$Na_2MoO_4 \cdot 2H_2O$	1.30 µg	1.14 µg	1.13 µg
$CuSO_4 \cdot 5H_2O$	1.70 µg	1.49 µg	1.48 µg
$CoSO_4.5H_2O$	0.91 µg	0.80 µg	0.79 µg
$NiCl_2 \cdot 6H_2O$	0.21 µg	0.19 µg	0.19 µg
Na_2SeO_3	24.74 ng	21.72 ng	21.47 µg
Thiamine HCl	0.77 µg	0.68 µg	0.67 µg
Biotin	0.27 ng	0.24 ng	0.23 ng
Vitamin B12	0.01 µg	0.01 µg	0.01 µg
$NaNO_3$	60.18 mg	53.58 mg	53.04 mg
$NaH_2PO_4 \cdot H_2O$	4.45 mg	3.96 mg	3.92 mg

Concerning the background system, these inputs include the processes for the production of the different chemicals required for the separation, the electricity used in the different stages (taken from the Belgian grid, with which the experiments where performed), the distribution of inputs up to the lab gate, laboratory supplies and equipment (flasks, electronic devices) and waste disposal. For the equipment, average weights and life spans were estimated according to manufacturers' specifications. With respect to transport, average distances of 800 and 600 km within continental Europe were considered for chemicals and materials, respectively. Waste transport distance was

estimated at around 50 km. Glass, steel and plastic wastes were assumed to be disposed in sanitary or inert landfills whereas the filter is sent to incineration. Inventory data for all those background processes were taken from Ecoinvent database, as summarized in **Table 2**.

Table 2. Summary of data sources for the background system

Type of involved process	Raw material/Energy	Data source
Chemicals	Soap	Ecoinvent database (Zah and Hischier, 2007)
	KCl	Ecoinvent database (Althaus et al., 2007)
	$NaHCO_3$	
	KBr	
	H_3BO_3	
	NaF	
	NaCl	
	Na_2SO_4	
	$MgCl_2 \cdot 6H_2O$	
	$CaCl_2 \cdot 2H_2O$	
	$SrCl_2 \cdot 6H_2O$	
	KI	
	$FeCl_3$	
	EDTA	
	$MnSO_4 \cdot 4H_2O$	
	$ZnSO_4 \cdot 7H_2O$	
	NH_4VO_3	
	$Na_2MoO_4 \cdot 2H_2O$	
	$CuSO_4 \cdot 5H_2O$	
	$CoSO_4.5H_2O$	
	$NiCl_2 \cdot 6H_2O$	
	Na_2SeO_3	
	Thiamine HCl	
	Biotin	
	Vitamin B12	
	$NaH_2PO_4 \cdot H_2O$	
	$NaNO_3$	Ecoinvent database (Frischknecht et al., 2007), completed with data from UNIDO/IFDC (1998) and Bhat et al. (1994)
Energy	Electricity (Belgian electricity profile)	Ecoinvent database (Dones et al., 2007)
Water	Tap water	Ecoinvent database (Althaus et al., 2007)
	Distilled water	

Table 2. Summary of data sources for the background system (*Cont.*)

Type of involved process	Raw material/Energy	Data source
Materials	HDPE	Ecoinvent database (Hischier, 2007)
	PP	
	PVC	
	Glass	
	Polyurethane foam	
	Silicon rubber	
	Lamps	Ecoinvent database (Hischier et al., 2007)
	Stainless steel	Ecoinvent database (Classen et al., 2007)
Transport	Truck 3.5-7.5 t	Ecoinvent database (Spielmann et al., 2007)
Waste treatment	Sanitary landfill	Ecoinvent database (Doka, 2007)
	Inert landfill	
	Electronic waste	
	Municipal incineration	

Allocation of the environmental burdens among the co-products

Since five specific compounds (i.e. myrcene, 10Z-bromomyrcene, 10E-bromo-3-chloromyrcene, apakaochtodene B and acyclic $C_{10}H_{14}Br_2$) were identified among the essential terpene oils extracted from *O. secundiramea* biomass, an allocation approach was needed to quantify the impacts associated with each of the main compounds. Due to the similar economic value and potential applications of the different terpenes, mass allocation is here proposed. The product distribution considered to allocate the environmental impacts was estimated from the composition measured by Polzin (2005) for the cultivation of *O. secundiramea* during 28 days with N-source excess.

Table 3. Partitioning fraction for mass allocation of the five main terpenes extracted from *O. secundiramea*

Bioactive compound	Fraction (%)
Myrcene	1.6
10Z-bromomyrcene	27.9
10E-bromo-3-chloromyrcene	25.1
Apakaochtodene B	8.5
Acyclic $C_{10}H_{14}Br_2$	36.9

4. Environmental impact assessment

The LCA was performed according to the ISO standards (ISO 14040, 2006). The environmental results associated with the production of essential terpene oils from *O. secundiramea* were quantified using the CML 2 baseline 2001 V2.05 method for the Life Cycle Impact Assessment (LCIA) (Guinée et al., 2002) with the following impact categories: abiotic depletion potential (ADP), acidification potential (AP), eutrophication potential (EP), global warming potential over a 100 year timeframe (GWP), ozone layer depletion potential (ODP), photochemical oxidants formation potential (POFP) as well as toxicity related impact categories of human toxicity (HTP), freshwater aquatic ecotoxicity (FEP), marine aquatic ecotoxicity (MEP) and terrestrial ecotoxicity (TEP).

The inventory data were implemented in the software SimaPro 8 to obtain the characterization results for the impact assessment (Goedkoop et al., 2013). The results for the three evaluated scenarios are summarized in **Table 4**.

Table 4. Environmental impact assessment results (characterization step) associated with the production of 700 mg essential terpene oils from *O. secundiramea* cultivated in an airlift PBR operated in 1 batch, 1 year and 10 years

Impact category	Unit	1 batch	1 year	10 years
ADP	kg Sb eq	0.49	0.42	0.41
AP	kg SO_2 eq	0.30	0.26	0.26
EP	kg PO_4^{-3}eq	0.13	0.11	0.11
GWP	kg CO_2 eq	70.58	61.24	60.45
ODP	kg CFC-11 eq	$1.51 \cdot 10^{-4}$	$1.50 \cdot 10^{-4}$	$1.50 \cdot 10^{-4}$
HTP	kg 1,4-DB eq	48.31	36.48	35.50
FEP	kg 1,4-DB eq	24.63	19.46	19.01
MEP	kg 1,4-DB eq	15.32	12.15	11.88
TEP	kg 1,4-DB eq	$1.71 \cdot 10^{-2}$	$1.61 \cdot 10^{-4}$	$1.55 \cdot 10^{-2}$
POFP	kg C_2H_4 eq	$1.25 \cdot 10^{-2}$	$1.06 \cdot 10^{-2}$	$1.05 \cdot 10^{-2}$

4.1. Effect of semi-continuous operation on the environmental profile

According to these results, the preparation of the culture medium (S2) and the inoculum (S3), which are the only stages affected by the change to semi-continuous operation, have a significant effect on the environmental profile for most impact categories. Thus, the implementation of a semi-continuous process in which the inoculum for each cultivation stage is obtained from a small fraction of the biomass from the previous culture represents a considerable improvement, mainly due to the reduction of electricity consumed in S3. For the semi-continuous process, the environmental burdens of the inoculum preparation at the start-up for one batch are calculated by dividing the total impact of S2 and S3 by the total number of subsequent batches that can be performed. Hence, their relative impact decreases when increasing the number of consecutive batches carried out with one single inoculum.

For a 1-year operation under these conditions, the characterization results are between 13% and 25% lower than those of a single batch, except for ODP and TEP, which displayed limited reductions around 1% and 6%, respectively. This is attributed to the low relative contribution of S3 to the aforementioned categories. When comparing the environmental profiles of 1 year and 10 years of semi-continuous operation, the differences are very limited in most categories (less than 4% for all impact categories). This finding demonstrates that the relative contribution of S3 is already very low compared to other stages in the 1-year scenario, so any further improvement is nearly negligible. The LCA results per category and particularly the relative contributions of all the stages and processes involved are further discussed in section 4.2.

4.2. Identification of hot-spots

Since the 1-batch scenario is not representative of a large scale process and the differences between 1-year and 10-year scenarios are very limited, the results provided in this section correspond to the semi-continuous operation for 1 year. Despite slight variations in the numerical values, the main findings and identified hot-spots are applicable to the three scenarios analyzed.

According to the results shown in **Figure 2a**, the cultivation stage (S4) is the main contributor to the environmental burdens derived from the production of essential terpene oils. The contributions range between 60% and 80% for all categories except for ODP and TEP. The contribution to ODP is dominated by the cell disruption stage (97%), whereas TEP is mainly due to cleaning (35%) and cell separation (38%), followed by cultivation (16%).

Electricity production is the main process responsible for the high contributions of the cultivation stage. As shown in **Figure 3**, it involves between 55% and 78% of the total

impact of S4, depending on the category. Moreover, the electricity required for the cultivation involves 86% of the total electricity consumption throughout the process. In particular, the electricity requirement for the illumination of the PBR corresponds to 81% of the total energy consumed, as depicted in **Figure 4**. The total electricity consumed throughout the process is responsible for 45-65% of the contributions to most categories, as shown in **Figure 2b**. Regarding other processes, the production of chemicals for the extraction is the most problematic issue in terms of ODP (97%), mainly due to the use of dichloromethane as solvent. The production of soap for cleaning (34%) and the materials (42%, mainly associated with the fabric filter) are important contributors to TEP. The production of materials also has other relevant contributions above 15% in categories such as AP, EP, FEP or MEP related to the manufacturing of lamps for reactor lighting. Among other secondary processes, only air supply has a noticeable impact in HTP (28%), FEP (17%) and MEP (16%). Although the carbon sequestration potential of algae during cultivation was considered, the benefit is very limited (<1%) in comparison with the total emissions of the process, mainly due to the high electricity consumption.

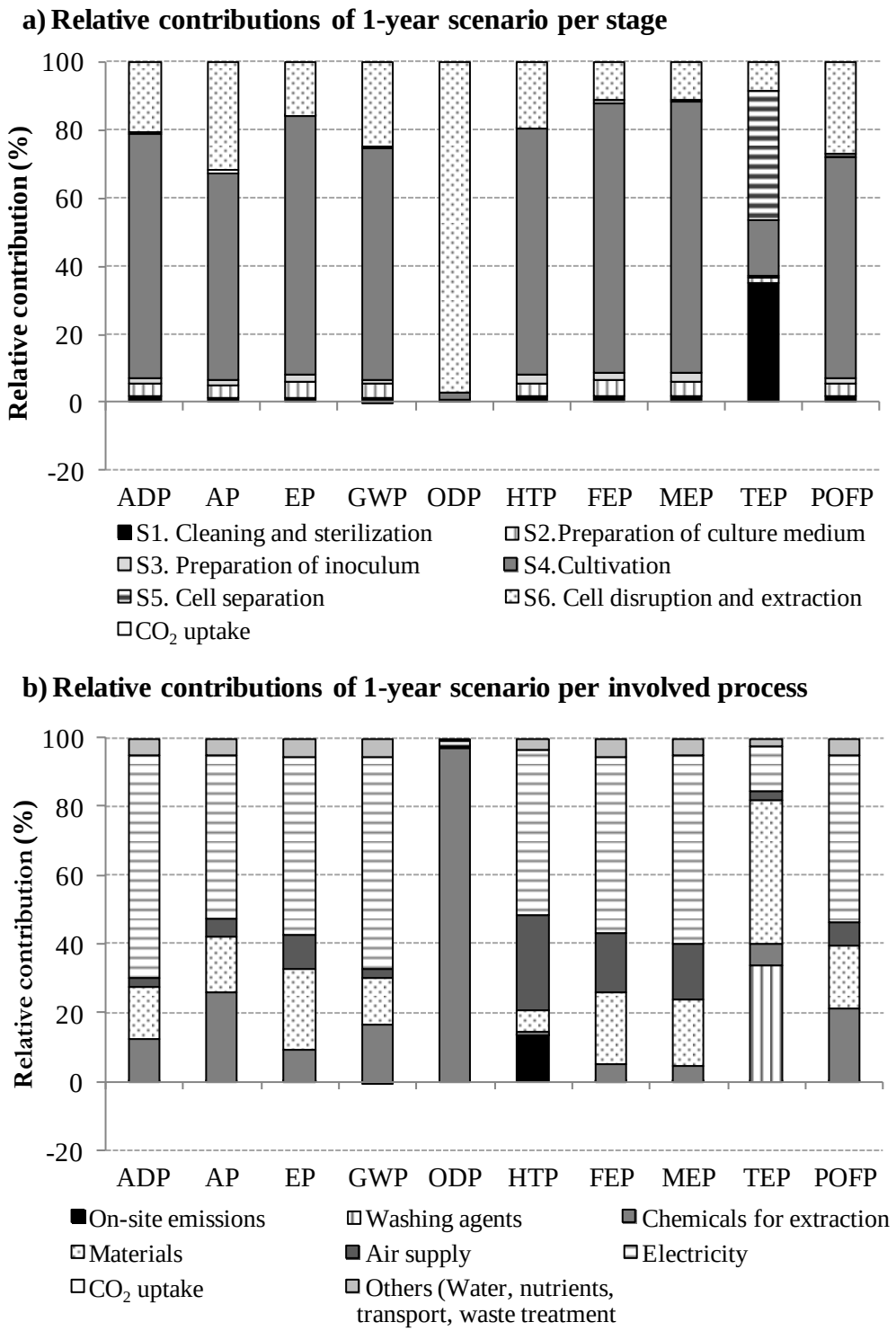

Figure 2. Relative contributions of the semi-continuous production of essential terpene oils by *O. secundiramea* to each impact category per a) stage and b) process involved.

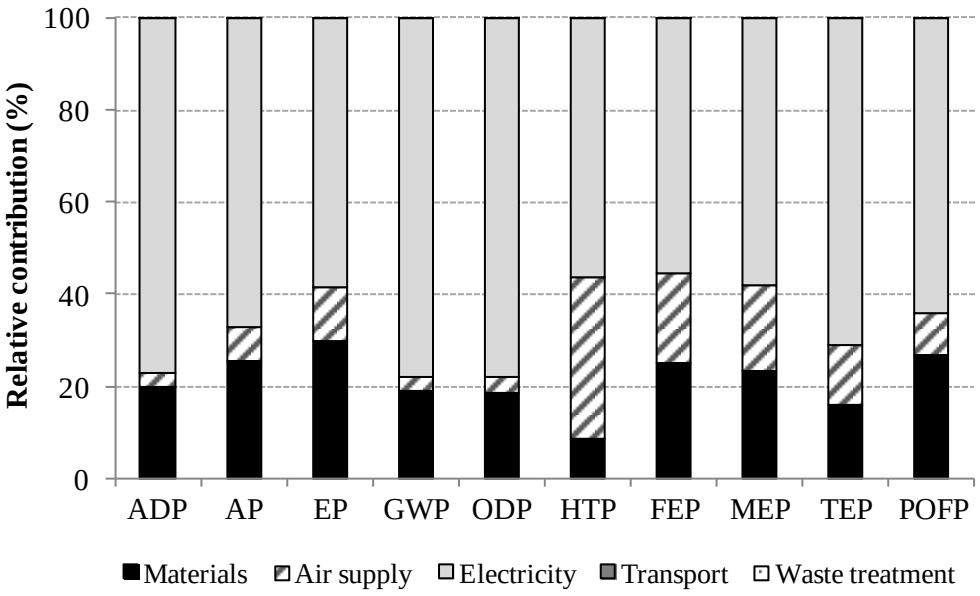

Figure 3. Relative contributions of the processes involved in the cultivation stage (S4) of the semi-continuous production (1 year) of essential terpene oils by *O. secundiramea*.

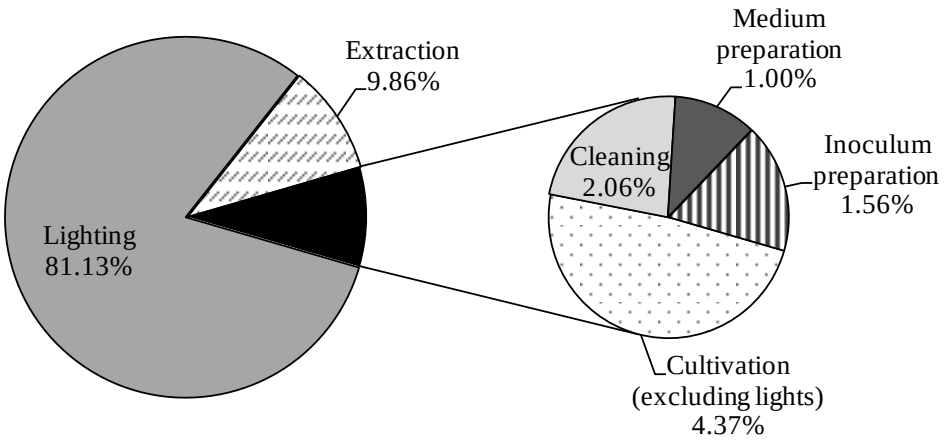

Figure 4. Relative contributions of the different steps to the total electricity requirements of the semi-continuous production (1 year) of essential terpene oils by *O. secundiramea*.

4.3. Contribution of the main terpenes

Table 5 shows the contribution of each product to the total environmental impact in each category according to the allocation procedure described in section 3. According to

this procedure, about 258 mg of acyclic $C_{10}H_{14}Br_2$ could be extracted after one algal culture, together with 195 mg of 10Z-bromomyrcene and 176 mg of 10E-bromo-3-chloromyrcene. Only 60 mg of apakaochtodene and 11 mg myrcene could be obtained. Since the acyclic $C_{10}H1_4Br_2$ terpene was the most abundant compound produced, it has the highest contribution to the environmental profile, according to the selected mass allocation approach. Thus, it is responsible for nearly 40% of the contributions with respect to the environmental impact of the total terpene oils. The compounds 10Z-bromomyrcene and 10E-bromo-3-chloromyrcene have similar contributions to each other, and their sum constitutes up to 80% of the remaining impacts. With regard to myrcene and apakaochtodene, their low content within the total extracted terpenes leads to very limited contributions to the total environmental impact.

Table 5. Environmental impact assessment results (characterization step) for each terpene from *O. secundiramea* in the 1-year scenario according to the proposed allocation approach

Impact category	Unit	Total impact (700 mg terpene oils)	Myrcene (11 mg)	10Z-bromomyrcene (195 mg)	10E-bromo-3-chloromyrcene (176 mg)	Apakaochtodene B (60 mg)	Acyclic $C_{10}H_{14}Br_2$ (258 mg)
ADP	kg Sb eq	0.42	0.01	0.12	0.11	0.04	0.16
AP	kg SO_2 eq	0.26	$4.20 \cdot 10^{-3}$	0.07	0.07	0.02	0.10
EP	kg PO_4^{-3} eq	0.11	$1.77 \cdot 10^{-3}$	0.03	0.03	0.01	0.04
GWP	kg CO_2 eq	61.24	0.98	17.09	15.37	5.21	22.60
ODP	kg CFC-11 eq	$1.50 \cdot 10^{-4}$	$2.41 \cdot 10^{-6}$	$4.20 \cdot 10^{-5}$	$3.78 \cdot 10^{-5}$	$1.28 \cdot 10^{-5}$	$5.55 \cdot 10^{-5}$
HTP	kg 1,4-DB eq	36.48	0.58	10.18	9.16	3.10	13.46
FEP	kg 1,4-DB eq	19.46	0.31	5.43	4.88	1.65	7.18
MEP	kg 1,4-DB eq	12.15	0.19	3.39	3.05	1.03	4.48
TEP	kg 1,4-DB eq	$1.61 \cdot 10^{-4}$	$2.58 \cdot 10^{-4}$	$4.49 \cdot 10^{-3}$	$4.04 \cdot 10^{-3}$	$1.37 \cdot 10^{-3}$	$5.94 \cdot 10^{-3}$
POFP	kg C_2H_4 eq	$1.06 \cdot 10^{-2}$	$1.70 \cdot 10^{-4}$	$2.97 \cdot 10^{-3}$	$2.67 \cdot 10^{-3}$	$9.05 \cdot 10^{-4}$	$3.93 \cdot 10^{-3}$

5. Discussion and recommendations

While microalgal cultivation processes have already been analyzed in detail as renewable energy sources (Brentner et al., 2011; Collet et al., 2011; Khoo et al., 2011;

Langlois et al., 2012; Lardon et al., 2009; Stephenson et al., 2010) and also for the production of high value biomolecules (Pérez-López et al., 2014a; 2014b), the few studies about the environmental effects of macroalgae focus on their use as energy sources ranging from direct combustion to gasification or anaerobic digestion (Aresta et al., 2005), biogas production being the most common route (Alvarado-Morales et al., 2013; Langlois et al., 2012; Pilicka et al., 2011).

Despite the lack of similar reports on macroalgae cultivation for the production of bioactive compounds, this study presents some findings consistent with previous work on micro and macroalgae. The cultivation stage was identified as the main contributor to the environmental impacts (Alvarado-Morales et al., 2013; Pérez-López et al., 2014a), linked to the high electricity requirements of the process (Aresta et al., 2005; Khoo et al., 2011; Pérez-López et al., 2014a). Other secondary processes, such as the production of chemicals for the extraction stage, were found to have a remarkable impact in specific categories, in accordance with LCA studies for microalgae (Pérez-López et al., 2014b). Due to the relevant contributions of the cultivation and extraction stages, two improved scenarios are proposed below. As in the previous section, the improvement is presented here for the 1-year scenario, since the behavior of the other cases is similar and no additional information is expected from their evaluation.

i) Energy optimization

The high electricity consumption in the cultivation stage was identified as the main hot-spot of the baseline 1-year semi-continuous process (Sc 1), in agreement with the findings of Pérez-López et al. (2014a) for microalgae. Thus, an optimization of the total energy required for the production of essential terpene oils is expected to have a great potential in the reduction of the environmental impacts. For this reason, three alternative scenarios involving a change in light regime are proposed. On the first alternative

scenario (Sc 2), a reduction of lighting from 14 h to 10 h is evaluated, whereas the second option (Sc 3) considers the effect of substituting the artificially-illuminated indoor PBR by an outdoor PBR with sunlight as the only source (a total value of 10 h daylight was estimated, according to average Belgian weather conditions). The third optimized scenario (Sc 4) takes into account a 50% reduction in electricity consumption of lights by substituting current lamps by light-emitting diodes (LEDs) (Pérez-López et al., 2014c). As highlighted by Pérez-López et al. (2014a), a reduction of illumination may lead to lower biomass productivity and final yield. Therefore, 10% reduction of final terpene oils per batch was estimated for the 10 h scenario, whereas 30% reduction was considered for the outdoor system. In the case of LEDs, the same yield as the baseline scenario was considered as a worst-case scenario according to results from Koç et al. (2013), who found that algal cultivation with LEDs was more efficient than conventional fluorescent lamps in terms of biomass yield. The changes in the LCI for Sc 4 with respect to the baseline 1-year scenario (shown in **Table 1**) are listed in **Table 6**. In the three scenarios, the incubation chamber for the preparation of inoculum in 500 mL flasks was eliminated, since the cultures proved their capability to be maintained in a controlled room at 23-25ºC with no power input thanks to the residual heat from the lighting. This change involved not only a reduction in the energy consumption, but also in the production of materials, transport and waste treatment for the corresponding equipment.

Table 6. Changes in the global inventory table for the production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR illuminated with LEDs instead of conventional lamps (FU=700 mg terpenes, equivalent to 1 batch)

	INPUTS from TECHNOSPHERE	
	Baseline 1-year semi-continuous mode	Sc 4. LEDs as light source
Materials		
S4. Cultivation		
Lamps	34.27 g	11.42 g
Transport		
S4. Cultivation		
Materials	58.08 kg·km	44.38 kg·km
Wastes	4.84 kg·km	3.70 kg·km
Energy		
S4. Cultivation		
Lighting	84.67 kWh	42.34 kWh
	OUTPUTS to TECHNOSPHERE	
Wastes to treatment		
S4. Cultivation		
Lamps	34.27 g	11.42 g

According to the results depicted in **Figure 5**, reductions of electricity consumption have a remarkable influence on the environmental profile. Although the benefits of Sc 2 (associated with the changing of light regime from 14 h to 10 h of artificial illumination) are limited, a 6% improvement can be achieved in categories such as ADP or GWP, whereas other reductions between 2% and 4% are obtained for other categories such as EP, FEP, MEP or POFP. However, a worse performance than that of the baseline case is observed for ODP and TEP, with impacts 11% and 7% higher. This is due to the estimated lower yield associated with the reduction in lighting hours, which leads to increased total contributions of other stages. Thus, ODP and TEP were the categories with the lowest contribution of electricity in the baseline scenario and were mainly due to other processes involved, such as the production of chemicals for the extraction (ODP) and the production of materials and washing agents (TEP). Due to the lower terpene oils content in the cultured biomass, the total environmental impact of the production of chemicals for extraction in the case of ODP and that of the filter

associated with cell separation (S5) increase by 18%. If the yield is maintained closer to the baseline scenario, the impact reductions would range between 11% and 16% for most categories (except for ODP and TEP).

Regarding Sc 3 (a total switch from indoors artificial illumination to outdoors sunlight), the significant reduction in electricity consumption results in considerably lower environmental impacts for most categories, despite the associated yield reduction. Excluding ODP and TEP, all categories show improvements ranging from 12% (AP or HTP) up to 32% (ADP). Again, ODP and TEP show higher environmental impacts in Sc 3 than in Sc 1. As explained for Sc 2, the reason is the lower yield caused by the lack of continuous artificial lighting, which results in a higher consumption of chemicals for S6 (affecting ODP) and a larger amount of materials and washing agents (affecting TEP) per FU.

Sc 4 (replacement of conventional lamps by LEDs) is the only option in which all categories show reductions of impact (between 0.7% and 26%), although they are slightly more limited for the specific categories of ADP and GWP than in Sc 3. However, since Sc 3 leads to significantly higher impacts to ODP and TEP, Sc 4 can be considered the most efficient in global terms. This is due to the possibility to maintain (or eventually improve) the biomass and terpenoids yields when using LEDs instead of conventional lamps.

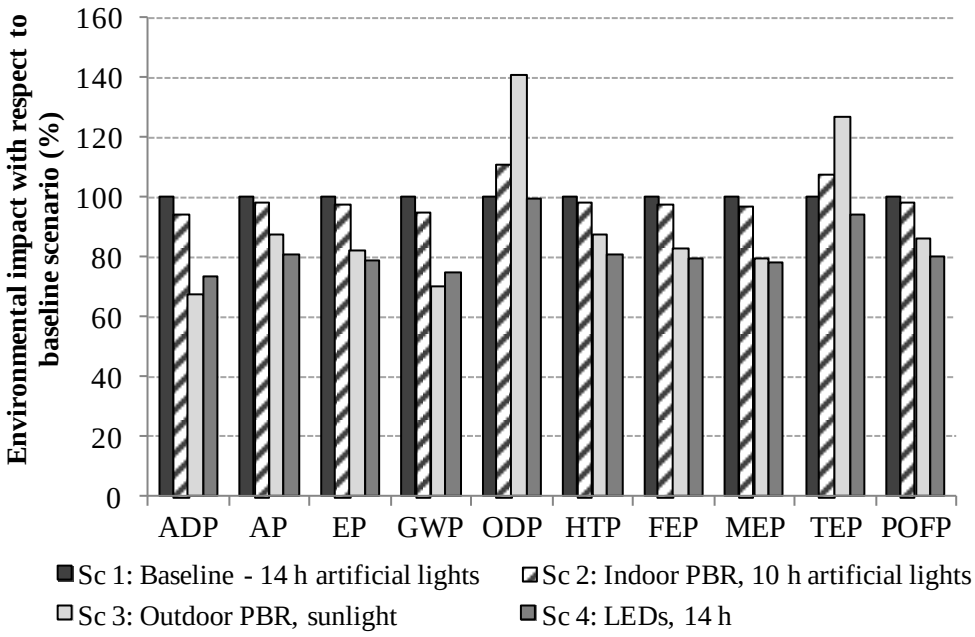

Figure 5. Effect of energy optimization on the environmental performance of the production of essential terpene oils by *O. secundiramea* considering a 4 h reduction of artificial lighting, solar illumination or lamps substitution by LEDs.

ii) Changes in downstream processes

Although the cell separation and extraction stages have limited secondary contributions to most impact categories, some relevant effects were found for specific cases, such as ODP and TEP. Therefore, an alternative scenario (Sc 5) is evaluated, including the following changes:

- Substitution of conventional cotton filter by organic cotton filter (i.e. cotton cultivated with no use of chemicals as pesticides).
- Substitution of current grinding procedure (with no electricity consumption) by a typical mechanical grinding stage, with an energy consumption of 0.54 kWh per FU estimated according to Pérez-López et al. (2014d).

- Optimization of solvent dose for the extraction with 20% reduction of initial requirement.
- Reduction of energy consumption for mixing after solvent addition by operating at the maximum capacity of the equipment.
- Alternative solvent evaporation method in chemical hood with no nitrogen gas addition.

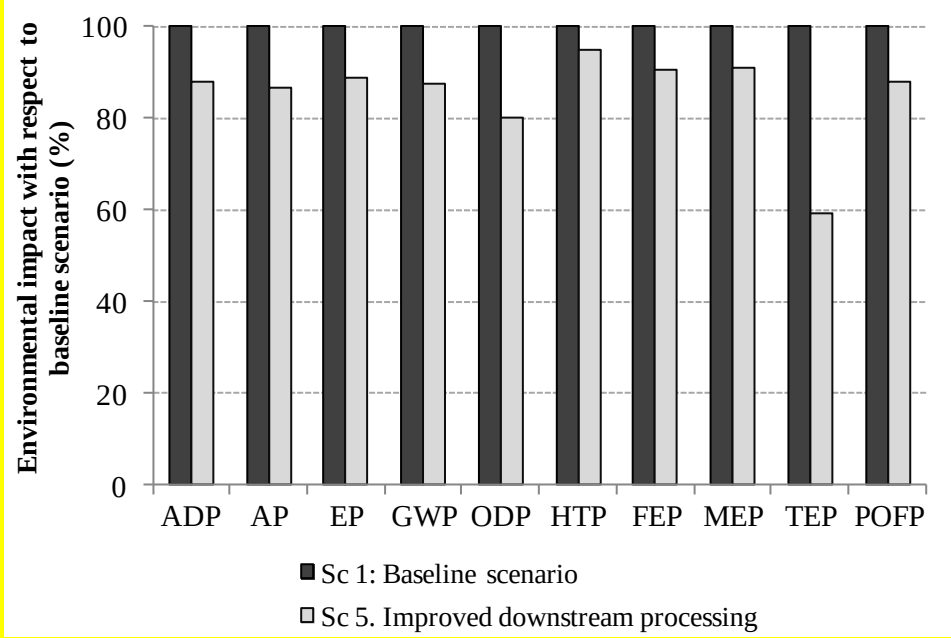

Figure 6. Effect of changes in downstream processing on the environmental performance of the production of essential terpene oils by *O. secundiramea*

According to **Figure 6Erreur ! Source du renvoi introuvable.**, the proposed changes may help to improve the environmental profile of the analyzed process in all categories. While the reduction associated with the proposed changes on downstream processing is between 5% and 15% for most impacts, contributions to ODP and TEP are remarkably affected. As expected, the change in the material of the filter allows a reduction of up to 40% of the total impact to TEP, whereas the reduction of chemicals for the extraction

(especially the 20% decrease for dichloromethane) is the main reason for the improvement in ODP.

iii) Combined optimized scenarios

Since the proposed changes can be applied together in an optimized scenario, the combination of improvement actions is globally evaluated below. The two analyzed scenarios are:

- Sc 6. Combined change in the lighting regime consisting of a 4 hour reduction of artificial lighting, together with the removal of the incubating cabinet for the preparation of the inoculum in 500 mL flasks and all the described changes in the downstream processing.
- Sc 7. Substitution of the indoor PBR with artificial lights by an outdoor PBR with sunlight and removal of the incubating cabinet for the preparation of the inoculum combined with the aforementioned optimized downstream processing.
- Sc 8. Combined substitution of conventional lamps by LEDs (14 h lighting regime maintained), removal of the incubating cabinet and optimized downstream processing.

As shown in **Figure 7**, the combined implementation of the suggested recommendations would allow important improvements in most impact categories. Sc 5, which considers the reduction of total lighting by 4 h, may lead to improvements ranging from 8% (for HTP) up to 40% (for TEP), being the contributions to most categories about 15-20% lower than in the baseline scenario. Sc 6, which includes the substitution of the current indoor PBR by an outdoor system illuminated with sunlight, involves remarkably higher reductions of impact in most cases, with improvements between 20% (HTP) and 50% (ADP). However, the reduction of final product yield associated with the use of sunlight

instead of controlled artificial lighting results in a higher impact of the cell disruption and extraction stage, particularly related to the increase in the required amount of dichloromethane per FU. Finally, the combination of LEDs in the cultivation stage with the removal of the incubation chamber and the optimized downstream processes may lead to significant improvements in all the categories that range between 20% (ODP) and 46% (TEP).

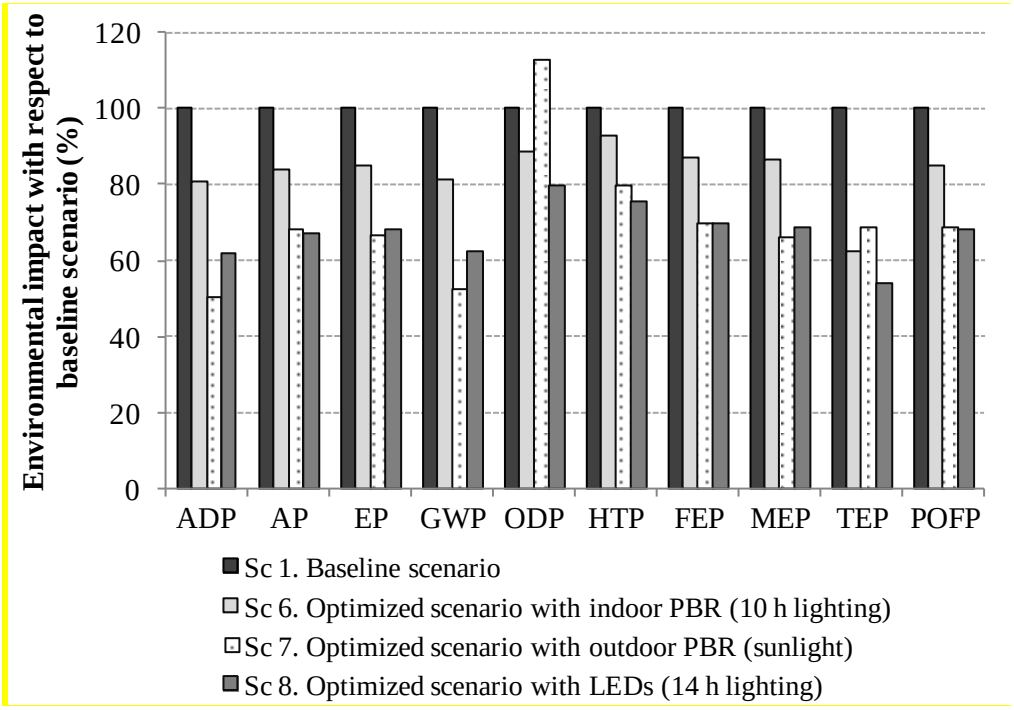

Figure 7. Environmental profile of the optimized scenarios (including energy reduction and improved downstream processing) for the production of essential terpene oils by *O. secundiramea*.

In addition to the proposed improvements, it should be noticed that the process involves the production of a large quantity of biomass that might be further used to obtain other co-products such as proteins, essential amino acids or carbohydrates (Gressler et al., 2011). The identification of additional bioactive compounds that could potentially be extracted from the remaining biomass would substantially help to improve the environmental performance of the production process. Moreover, the remaining

biomass after high-value compounds extraction could be applied in an anaerobic digestion process to recover energy and produce fertilizers (Collet et al., 2011), although the environmental benefits obtained in other LCA studies of bioactive compounds associated with this recovery process are limited due to the high impact of the cultivation and extraction stages (Pérez-López et al., 2014b). Thus, the development of a process for the efficient use of macroalgal biomass may require the combined optimization of energy-intensive stages, linked to high environmental burdens, with the maximization of co-product recovery that contributes to reduce the individual impact of each obtained fraction.

6. Conclusions

This study aims to provide the first life cycle inventory and impact assessment results for macroalgae cultivation and extraction applied to the production of high value bioactive molecules with applications in strategic sectors such as pharmaceutical or nutraceutical industries. The evaluation of the production of valuable compounds by *O. secundiramea* cultivated in an airlift PBR allowed the identification of the major environmental concerns associated with the system. As for microalgal processes, the cultivation in the reactor constitutes a major environmental concern, linked to the high electricity consumption. Other involved processes, such as materials or chemicals associated with biomass separation and extraction have relevant contributions in specific categories. In addition, the implementation of semi-continuous operation has proved to involve significant environmental benefits due to the reduction in the contributions of some stages that only need to be performed at the start-up.

Moreover, LCA was applied to evaluate potential improvements with respect to the baseline case, according to the identified hot-spots. Thus, two scenarios were proposed in order to reduce the total electricity consumption throughout the process and another option was evaluated for the improvement of downstream processing stages. The alternative scenarios were combined and resulted in two optimized processes with an improved environmental profile.

The design of novel processes for the exploitation of renewable resources requires objective supporting tools to evaluate their sustainability from environmental, economic and social perspectives. To this end, LCA methodology was successfully applied in this paper as an optimization tool to provide strategic information for the future implementation of macroalgal bioactive compounds on a commercial scale.

Acknowledgements This project has been developed within the framework of the European Project BAMMBO (FP7 KBBE-2010-4-265896) and has been partially financed by the Xunta de Galicia (Project Reference GRC 2010/37). P. Pérez-López would like to thank the Spanish Ministry of Education for the research scholarship (AP2012-1605).

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