



Assessing the Environmental Performance of a New Biotechnological Sensor for Air Quality Based on Devitalised Moss Clone

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In recent years, mosses have been suggested as feasible biomonitors for the control of air quality. The most suitable type of biosensor consists of moss transplants that can be either harvested from unpolluted natural habitats or cultivated in photobioreactors. The production and devitalisation of moss was assessed with the aim of identifying the stages of the process with potential environmental impacts. The use of energy, especially associated with refrigeration in the cultivation stage, was the main factor contributing to the environmental impacts considered, ranging from 85% of the impact in marine eutrophication up to 95% for the rest of categories. Results were compared with previous studies dealing with algae production in photobioreactors, in which the electricity use for lighting also constituted a major hotspot. Scenarios based on reduced energy demand, and implementing alternative sources were proposed and showed better environmental profiles than the baseline scenario. Particularly, the use of photovoltaic energy could reduce the impacts by 50% in the analysed categories, except for terrestrial ecotoxicity, with significantly lower improvement ratios if photovoltaic energy was partially combined with conventional energy. The option of optimising the refrigeration system also provided significant reductions, ranking as the best alternative when terrestrial ecotoxicity was assessed.

Keywords: air quality, environmental assessment, life cycle assessment (LCA), moss clone, photobioreactor.

1 Introduction

Atmospheric pollution has increasingly shown adverse effects on both, human health and environment (Kampa & Castanas, 2008). Europe has become aware of the problem and European countries deal with atmospheric pollution by assessing, monitoring and controlling air quality (Garrido, Jiménez-Guerrero, & Ratola, 2014; Vallero, 2014). Recently, compounds such as

polycyclic aromatic hydrocarbons (PAHs) and trace metals have emerged as one of the most important issues regarding air quality. For this reason, their monitoring is recommended by EU legislation (European Commission (EC), 2004). Typically, conventional techniques such as bulk deposition collectors for particles and gaseous samplers have been applied for air quality measurements.

Nevertheless, automatic devices at monitoring stations show technical difficulties and high economical costs when micro-contaminants are measured. The use of mosses as biomonitors of atmospheric quality has arisen to overcome the limitations associated with traditional techniques (Rühling & Tyler, 1968; Gerdol, Marchesini, Iacumin, & Brancaloni, 2014; Vuković et al., 2014; Wu, Wang, & Zhou, 2014). The main advantage of using mosses as biomonitors is their high efficiency to accumulate a wide range of airborne pollutants, including trace metals (Schröder et al., 2008; Fabure et al., 2010) and organic compounds (Harmens, Foan, Simon, & Mills, 2013).

The development of biological monitoring methods based on mosses can be classified into passive biomonitoring, when endogenous species are used (Ferreira, Santos, Souza, Júnior, & Alves, 2012), and active biomonitoring, based on placing biosensor transplants into the area under assessment. This alternative offers a number of advantages over the former option (Aboal, Couto, Fernández, & Carballeira, 2006; Ares et al., 2012, Vuković et al., 2014). Moss bags consist of external structures containing moss inside and they are the most common system of active biomonitoring due to their reliable results, simplicity and cost effectiveness (Tretiach et al., 2007; Cao et al., 2009). The moss used in active biomonitoring can be either harvested from unpolluted natural habitats and cleaned before its use or alternatively, it can be cultured in photobioreactors. Photobioreactors allow cultivating moss from an isolated moss clone and producing a standard culture (Hohe & Reski, 2002). The process of moss cultivation in photobioreactors starts by adding the inoculum obtained in agitated flasks. Once the reactor is inoculated, biomass growth with high values of yield and productivity is possible with a simple medium of inorganic salts, along with illumination and carbon dioxide as energy and carbon sources (Reski & Abel, 1985). It is a simple process that does not need the addition of more complex additives other than nutrients for the culture medium.

In general, moss bioreactors are designed at laboratory scale as stirred glass vessels or modular glass tubular reactors. Although the former have been widely used for submerged cultures (Decker & Reski, 2004; 2007), limited illumination is inherent to this reactor configuration. As a consequence, tubular reactors are highly recommended for scaling up (Lucumi, Posten, & Pons, 2005; Lucumi & Posten, 2006; Decker & Reski, 2007; Perner-Nochta, Lucumi, & Posten, 2007). Optimal conditions for moss cultivation in photobioreactors comprising pH, nutrients, external illumination, stirring speed, aeration and density of inoculation have been established for the species *Physcomitrella patens* (Hohe & Reski, 2002; Schween, Hohe, Koprivova, & Reski, 2003; Hohe & Reski, 2005). Glass photobioreactors ranging from 10 to 30 l with external illumination, e.g. normal fluorescent tubes (Decker & Reski, 2004) or white light metal halide

lamps (Lucumi & Posten, 2006), have been successfully applied for moss cultivation-

Regarding the cultivation of moss in bioreactors for air quality control, *Pseudoscleropodium purum* and *Ceratodon purpureus* have been cultivated with the objective of accumulating metal trace elements (Fabure et al., 2010) and *Sphagnum palustre* has been recommended as the most suitable species to capture and accumulate contaminants (Ares et al., 2012).

Recent studies highlighted the advantages of using devitalised moss for active biomonitoring (Hohe & Reski, 2005): the avoidance of moss growth during exposure time,—the possibility to provide results that are not affected by the metabolic activity (Fernández et al. 2009, Adamo et al. 2011) and the replicability of the obtained results. Oven-drying procedure was found to be the most adequate alternative to devitalise moss since this option maintains moss structure after drying (Lucumi et al. 2005; Fernández, Ares, Rey-Asensio, Carballeira, & Aboal, 2009).

With the objective of evaluating all the environmental impacts associated with products, processes and services, life cycle assessment (LCA) has been considered as the most suitable methodology (ISO 2006). Biomass cultivation in photobioreactors has only been assessed for algae while there is no previous study for moss photobioreactors. The main outcomes of these studies revealed the importance of the photobioreactor operation in the global environmental impact (Pérez-López et al., 2014b; Pérez-López et al., 2014c; Pérez-López et al., 2014d).

This research paper assesses the production process of the moss species *Sphagnum palustre* in a photobioreactor as well as its further devitalisation. The process consisted of several steps including preparation of the culture medium, cultivation of the inoculum, production of moss in a 15 l photobioreactor and finally, the stages of washing and devitalisation of the moss. Additionally, cleaning with water and soap and sterilisation of the reactor were included into the analysis. The outcomes will identify the stages with the largest environmental impacts and different options will be proposed to improve the environmental performance of moss cultivation.

2 Materials and methods

2.1 Goal and scope definition

This paper aims at performing the environmental assessment of the production process of the moss *Sphagnum palustre* in a photobioreactor at laboratory scale. The objective of the study was to determine the most relevant stages from an environmental point of view. The process was evaluated according to a cradle-to-gate perspective, including from the production processes, such as chemicals and energy production, to the final product: the moss clone. The functional unit was

defined as 109 g of dry moss, which corresponded to 1 batch of production.

2.2 Overview of the production system

The system for the production of devitalised moss was split in five subsystems (Figure 1)

according to the different procedures carried out during the process: Subsystem 1, reactor cleaning; Subsystem 2, preparation of the culture medium; Subsystem 3, cultivation of the inoculum; Subsystem 4, cultivation of the moss and Subsystem 5, washing and devitalisation of the moss.

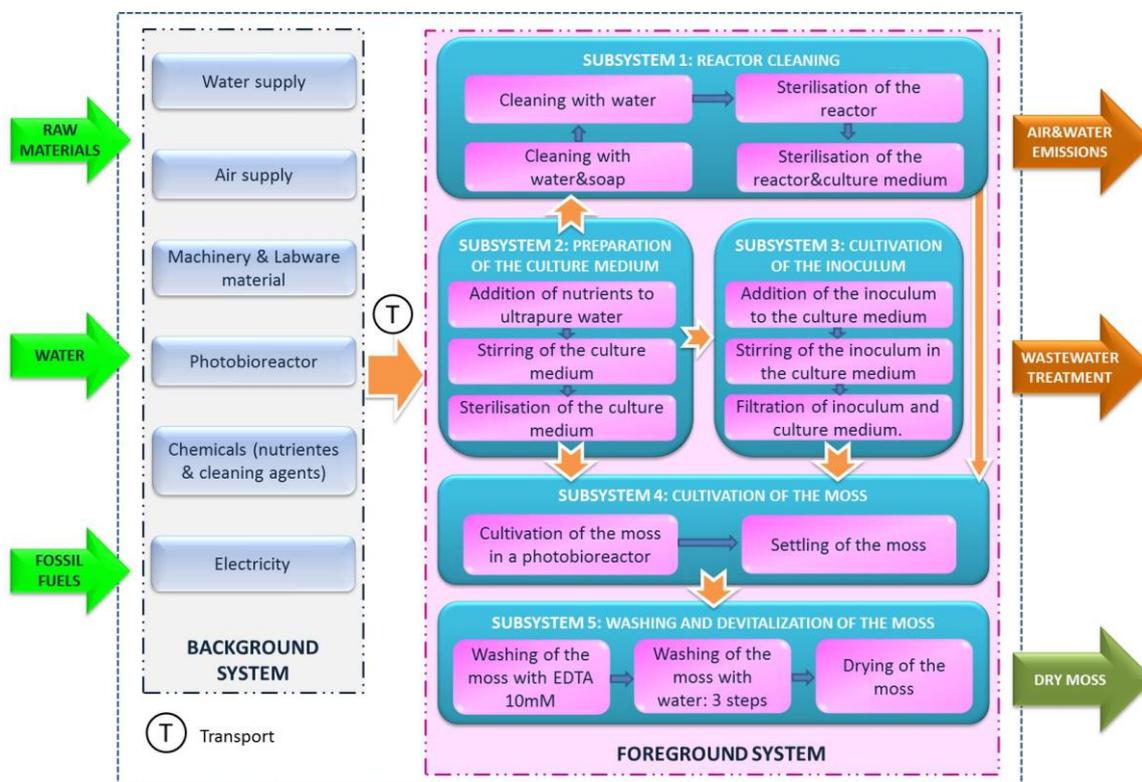


Figure 1. Detailed description of system boundaries for the production system of devitalised moss.

Subsystem 1. Reactor cleaning: The reactor was cleaned with 75 l of water and liquid soap, followed by a washing step with 5 l of distilled water. Thereafter, it was autoclaved twice for 20 min (1 atm, 121 °C); in the second step, filled with 2 l of culture medium.

Subsystem 2. Preparation of the culture medium: The composition of the culture medium comprised the following chemicals for a total volume of 13 l: 3.25 g KH_2PO_4 , 3.25 g KCl, 3.25 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 13.00 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.16 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $4.02 \cdot 10^{-2}$ g H_3BO_3 , 0.11 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, $5.40 \cdot 10^{-3}$ g KI, $5.60 \cdot 10^{-2}$ g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $1.57 \cdot 10^{-3}$ g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, $1.63 \cdot 10^{-4}$ g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $1.90 \cdot 10^{-4}$ g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 1.30 g NH_4NO_3 , 296.66 g $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ and $2.67 \cdot 10^{-3}$ ml HCl. A value of pH 4 was adjusted by adding HCl (445 μl , 0.5 N). The next step was the sterilisation of culture medium (13 l) in autoclave for 20 min (1 atm, 121 °C), to be used as follows: 2 l for Subsystem 1, 1 l for inoculum cultivation in Subsystem 3 and the remaining 10 l that were directly used for the start-up of the reactor in Subsystem 4.

Subsystem 3. Cultivation of the inoculum: Small-scale moss cultivation was performed for inoculum growth. For this purpose, 5 flasks of 500 ml were inoculated with 0.36 g dry weight (dw), equivalent to 18 g fresh weight (fw), per flask under

constant stirring and artificial illumination with a 16:8 regime (36 W) for 21 days. Once the required density was achieved: 0.8 g dw (40 g fw) per flask, the culture medium was filtered and the moss was ready to inoculate the photobioreactor.

Subsystem 4. Cultivation of the moss: The 15 l glass photobioreactor (Figure 2) previously autoclaved with 2 l of culture medium (in Subsystem 1) was inoculated with 4 g dw (200 g fw) of inoculum from Subsystem 3 in 10 l of culture medium prepared in Subsystem 2. The photobioreactor was illuminated with continuous light at $210 \mu\text{mol m}^{-2} \text{s}^{-1}$ with 14 LED tubes of 8 W each. The moss was continuously aerated with 2 l min^{-1} of air with 2% CO_2 and agitated at 500 rpm for 1 min every day. Temperature was set at 25 °C using a refrigerated bath. Water was kept at 10 °C inside the refrigerated bath, which was used not only to maintain the culture medium at 25 °C but also for the condenser to avoid evaporation of the culture medium. In general, pH was naturally kept in a range between 3 and 4 without the addition of chemicals, which favoured moss growth.

After 27 days of operation, the moss production achieved a value of $3.9 \text{ g dw day}^{-1}$ (Beike et al. 2014). The culture medium was poured off to collect moss: 109 g dw (1,400 g fw) with 100% moisture.



Figure 2. Stirred glass tanks operated for *S. palustre* cultivation after 1 week (left) and 3 weeks (right) from inoculation. Photographs courtesy of BioVia Consultor Ambiental.

Subsystem 5. Washing and devitalisation of the moss: The moss was washed for 20 min with 8.7 l of 10 mM ethylenediaminetetraacetic acid (EDTA), following a ratio of 1 l of EDTA per 12.5 g dw. Thereafter, 3 consecutive washes of 20 min with 10.93 l of distilled water in each step were carried out, according to a ratio of 1 l of distilled water per 10 g dw. The moss was washed with the objective of removing ions attached to the tissue, which would hinder moss accumulation capacity. Finally, devitalisation was performed in an oven, with a temperature slope rise every 8 h: 8 h at 50 °C, 8 h at 80 °C and 8 h at 100 °C, for a final weight of 109 g of dry biomass.

2.3 Life cycle inventory (LCI)

The LCI data for the foreground system were obtained from on-site measurements whereas background data were taken from the Ecoinvent database (Ecoinvent, 2007) (Table 1). The latter includes the production of chemicals such as nutrients and EDTA as well as soap added to clean the reactor, water and air supply, energy used, equipment and laboratory ware such as glass beakers and test tubes, transport and waste treatment scenarios. The inventory for energy production was modified according to data from the electricity production/importation system for Spain in 2013 (RedEléctrica, 2013). The electricity transmission network, emissions of sulphur hexafluoride to air as well as losses during the low voltage transmission and transformation from high to low voltage were considered (Dones et al., 2007).

Table 1. Summary of data sources considered in this study.

Inventory inputs		Data sources
Energy	Electricity (Spanish profile)	Ecoinvent database (Dones et al., 2007)
Materials	Polypropylene	Ecoinvent database (Hischier, 2007)
	White glass	
	Amber glass	
	Steel	Ecoinvent database (Steiner & Frischknecht, 2007)
	Aluminium	
	Parafilm	Ecoinvent database (Althaus et al., 2007)
Chemicals	KH ₂ PO ₄	Ecoinvent database (Althaus et al., 2007)
	KCl	
	MgSO ₄ ·7H ₂ O	
	Ca(NO ₃) ₂ ·4H ₂ O	
	FeSO ₄ ·7H ₂ O	
	H ₃ BO ₃	
	MnSO ₄ ·H ₂ O	
	KI	
	ZnSO ₄ ·7H ₂ O	
	Na ₂ MoO ₄ ·2H ₂ O	
	CuSO ₄ ·5H ₂ O	
	Co(NO ₃) ₂ ·6H ₂ O	
	NH ₄ NO ₃	
	C ₁₂ H ₂₂ O ₁₁	
	HCl	
EDTA		
Cleaning agent	Soap	Ecoinvent database (Zah & R., 2007)
Water	Tap water	Ecoinvent database (Althaus et al., 2007)
Transport	Lorry, 16-32 t, EURO 4	Ecoinvent database (Spielmann, Bauer, Dones, & Tuchschnid, 2007)
Water treatment	WWTP	Ecoinvent database (Doka, 2007)

The global LCI of the production process is shown in Table 2. All nutrients used to prepare the culture medium were inventoried and compounds that were not included in the Ecoinvent database were considered as chemicals with comparable production processes. The average transport distance of 20 km was assumed for the transportation of nutrients from the manufacturing industry to the considered process location. The culture medium poured off in Subsystems 3 and 4 was discharged to the sewage

system for its further treatment in a wastewater treatment plant (WWTP). The same scenario was assumed for water and soap used as cleaning agents in Subsystem 1. On the contrary, the direct discharge to aquatic environments was assumed for EDTA used in Subsystem 5 based on the negligible removal found for this compound in conventional WWTPs (Clara et al., 2012). Regarding air emissions, only 5.5% of the CO₂ supplied to the system was used by the moss and the excess of CO₂ was released to the atmosphere.

Table 2. Global inventory for the production of moss clone in a 15 l stirred glass photobioreactor (Functional unit: 109 g of moss).

Inputs from nature			
Materials			
Subsystem 3. Cultivation of the inoculum			
Moss	1.80 g		
Inputs from technosphere			
Materials		Materials	
Subsystem 1. Reactor cleaning		Subsystem 4. Cultivation of the moss	
Potable water	75.00 l	Compressed air (2% CO ₂)	77.76 m ³
Distilled water	5.00 l	Photobioreactor, glass	103.56 g
Soap	2.00·10 ⁻² ml	Laboratory plastic ware	17.28 g
Subsystem 2. Preparation of the culture medium		Stainless steel	2.47 g
Ultrapure water	13 l	Photobioreactor, stainless steel	103.56 g
KH ₂ PO ₄	3.25 g	Steel	59.18 g
KCl	3.25 g	Silicone	176.45 g
MgSO ₄ ·7H ₂ O	3.25 g	Subsystem 5. Washing and devitalisation of the moss	
Ca(NO ₃) ₂ ·4H ₂ O	13.00 g	Ultrapure water	41.53 L
FeSO ₄ ·7H ₂ O	0.16 g	EDTA	32.55 g
H ₃ BO ₃	4.02·10 ⁻² g		
MnSO ₄ ·H ₂ O	0.11 g	Electricity	
KI	5.40·10 ⁻³ g	Subsystem 1. Reactor cleaning	
ZnSO ₄ ·7H ₂ O	5.60·10 ⁻² g	Autoclaving	5.20·10 ⁻² kWh
Na ₂ MoO ₄ ·2H ₂ O	1.57·10 ⁻³ g	Subsystem 2. Preparation of the culture medium	
CuSO ₄ ·5H ₂ O	1.63·10 ⁻⁴ g	Ultrapure water system	0.52 kWh
Co(NO ₃) ₂ ·6H ₂ O	1.90·10 ⁻⁴ g	Stirring	0.11 kWh
NH ₄ NO ₃	1.30 g	Autoclaving	0.17 kWh
C ₁₂ H ₂₂ O ₁₁	296.66 g	Subsystem 3. Cultivation of the inoculums	
HCl	2.67·10 ⁻³ ml	Lighting	4.03 kWh
Laboratory glassware	12.59 g	Stirring	6.05 kWh
Glass amber bottles	1.61·10 ⁻² g	Subsystem 4. Cultivation of the moss	
Laboratory plastic ware	157.05 g	Reactor tower	47.23 kWh
Stir bars	2.12·10 ⁻⁴ g	Refrigerated bath	139.30 kWh
Caps	1.10 g	Air blowing	2.99 kWh
Crêpe paper	1.00 g	Lighting in photobioreactor	72.58 kWh
Subsystem 3. Cultivation of the inoculum		Subsystem 5. Washing and devitalisation of the moss	
Laboratory glassware	0.15 g	Ultrapure water system	1.84 kWh
Laboratory plastic ware	17.41 g	Stirring	8.00·10 ⁻² kWh
Rubber caps	5.64·10 ⁻³ g	Drying	10.91 kWh
Stainless steel	6.88·10 ⁻³ g		
Polypropylene caps	1.99·10 ⁻³ g	Transport	
Crêpe paper	0.55 g	Subsystem 2. Preparation of the culture medium	
Fabric	0.11 g	Lorry, 16-32 t, EURO 4	6.42 kg·km
Nitrile gloves	13.14 g		
Aluminium foil	77.50 g		
Parafilm	3.12 g		
Outputs to technosphere			
Liquid waste to treatment		Liquid waste to treatment	
Subsystem 1. Reactor cleaning		Subsystem 4. Cultivation of the moss	
Cleaning agent	80.02 l	Culture medium	11.89 l
Subsystem 3. Cultivation of the inoculum		Subsystem 5. Washing and devitalisation of the moss	
Culture medium	1.00 l	Water	41.64 l

Outputs to environment			
Air emissions		Water emissions	
Subsystem 4. Cultivation of the moss		Subsystem 5. Washing and devitalisation of the moss	
Air (excluding CO ₂)	76.29 m ³	EDTA	32.55 g
CO ₂	1.47 m ³		

2.4 Life cycle impact assessment (LCIA)

The analysis of the midpoint environmental impacts was performed by using ReCiPe (Goedkoop et al. 2009). The impact categories considered according to the ReCiPe methodology were: climate change (CC), ozone depletion (OD), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), human toxicity (HT), photochemical oxidant formation (POF), terrestrial ecotoxicity (TET), freshwater ecotoxicity (FET) and marine ecotoxicity (MET). SimaPro version 8 was the software selected to perform the LCA calculations (<http://www.pre-sustainability.com/simapro>).

3 Results and discussion

3.1 Impact categories

Figure 3 shows the relative contributions per subsystem to each impact category. Subsystem 4, related to the cultivation of moss in photobioreactor, was identified as the most relevant stage in terms of environmental burdens, representing between 78.9% and 91.1% of the total impact for ME and HT, respectively. Regarding the other subsystems, reactor cleaning (Subsystem 1) and preparation of culture medium (Subsystem 2) were identified as the least significant contributors. The former led to around 7.4% in ME while the highest contribution for Subsystem 2 was 1.4% in POF category. Regarding Subsystem 3, the highest relative contribution was observed in OD category (around 7.3%) where laboratory ware and electricity production led to 50%

each. Finally, Subsystem 5 caused 5% of the total impact to each category, except for ME, in which this subsystem contributed 9.6% to the total impact.

The issues comprised in each subsystem were analysed separately and energy was found to be the main contributor to all environmental categories (Figure 4). More specifically, energy required for the refrigerated bath (139.30 kWh per batch) represented more than 50% of the total energy required in the production system. Consequently, the energy used in the refrigerated bath could be considered as the hotspot of the studied process, and therefore, temperature set at 10°C inside the bath is considered susceptible of revision. A similar profile was obtained when the process for eicosapentaenoic acid (EPA) extraction from microalgae was analysed (Pérez-López et al., 2013). The authors demonstrated that the cultivation of the microalgae *Phaeodactylum tricornutum* in photobioreactors contributed more than 80% to the total impact, which is in accordance with the outcomes from this study. Regarding the importance of energy use, it was underlined that energy is the main hotspot in the microalgae cultivation process, external lighting being the main source since no refrigeration system was used in the microalgal photobioreactor system (Pérez-López, Feijoo, & Moreira, 2014a; Pérez-López et al. 2014c). Additionally, the importance of energy use in artificially illuminated systems was determined for the cultivation of marine sponges in aquariums, in which case the aquarium lighting accounted for three fourths of the total electricity requirements (Pérez-López et al. 2014e).

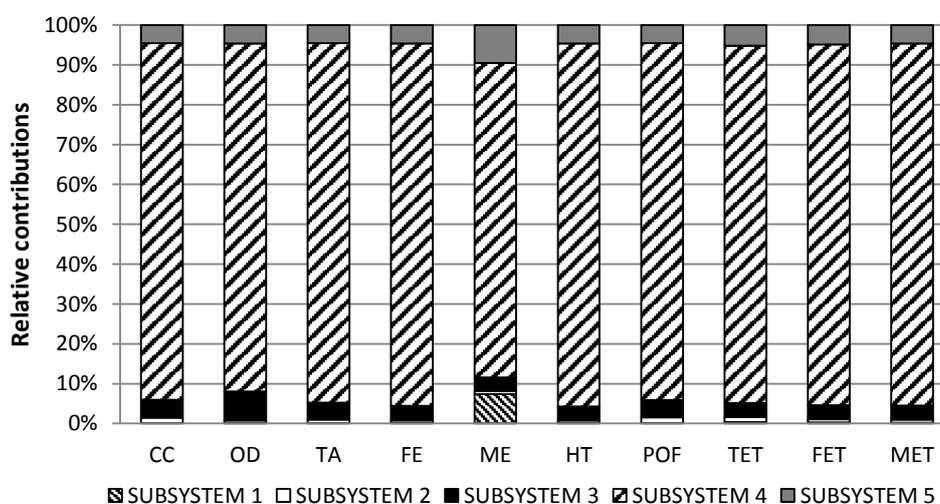


Figure 3. Relative contributions per subsystem to each impact category (in %).

In CC impact category, energy led to 88.07 kg CO₂ eq per FU (97% of the total value of 90.44 kg CO₂ eq per FU), where the refrigerated bath used in

Subsystem 4 contributed with 49%. The analysis of the Spanish energetic profile shows the dependence on fossil fuels, specifically, electricity based on coal

contributes up to 15%. In this case, the extraction of hard coal from mine was the main responsible of carbon dioxide emissions.

Regarding OD, electricity represented more than 95% of the total impact ($7.31 \cdot 10^{-6}$ kg CFC-11 eq per batch) while laboratory ware only accounted for a minimal contribution: 4% (Figure 4). Emissions of

Halon 1211 to air through natural gas transportation were responsible for most of the OD impact associated to energy. Regarding laboratory ware, most of the impact corresponded to the production of aluminium foil used in Subsystem 3. In this case, Halon 1301 was emitted to the atmosphere associated with aluminium foil production.

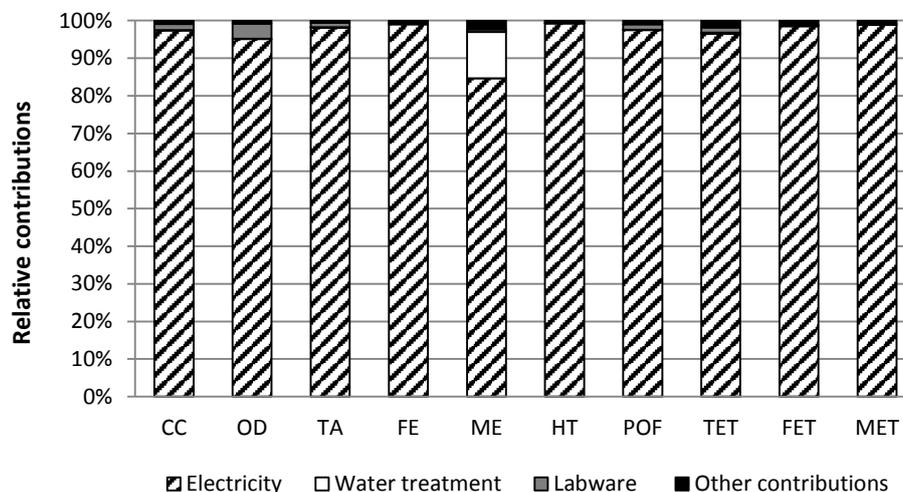


Figure 4. Contributions from the different input parameters to each impact category (in %).

The production of electricity was the main contributor (98%) to TA associated impact: 0.65 kg SO₂ eq per FU (Figure 4). Most of energy use corresponded to the use of refrigerated bath in Subsystem 4. In this case, laboratory ware production only accounted for 1% of the environmental burdens. The rationale behind SO₂ emissions to air is the production of electricity in coal based power plants.

Subsystem 4 led to 91% of the FE total impact ($4.18 \cdot 10^{-2}$ kg P eq per FU), followed by Subsystems 5 and 3. On the other hand, Subsystems 1 and 2 presented negligible contributions (Figure 3). Energy use accounted for 99% of the total burdens (Figure 4). The main cause for this impact was associated to the phosphate emitted to water during the extraction of lignite and hard coal from mines. ME showed relevant differences in the other categories. The category was the least influenced by energy use, although it represented around 85% of the total impact. Emission of nitrogen oxides to air in hard coal burning had significant relevance in the impact associated to electricity. In this case, the treatment of liquid emissions in a WWTP accounted for 12% of the total impact. Most liquid emissions are generated in Subsystem 1, due to the significant volume of water (80 l) used for reactor cleaning and consequently, around 50% of the impact associated to further treatment of wastewater corresponded to Subsystem 1. Nitrogen emissions during WWTP construction were the main factor responsible for the impact. Similarly to CC, TA and FE, the categories POF, HT, FET and MET showed significant impacts due to the contribution of emissions from the production of energy which was used during the process (around 99% of the total impact). Emissions of Mn to water and NO_x to air led to high impacts in HT and POF

and Ni emitted to water mainly contributed to FET and MET impacts.

Although TET showed a similar behaviour with energy as the main hotspot, not only hard coal, but also oil and photovoltaic energy led to TET associated impacts. In this case, Ag and V emissions released to air contributed to photovoltaic and oil energy associated impacts, respectively.

3.2 Improvement scenarios

Energy use was identified as the main hotspot in the moss production system. Particularly, the supply of energy by non-renewable sources such as hard coal appeared to be the least recommendable options under an environmental perspective. Therefore, different scenarios were proposed with the aim of studying alternative energy sources and reducing energy requirements. The base scenario, which corresponds to the real case study, was named as Scenario A. Scenario B corresponded to the combination of two energy sources: electricity from the grid (50%) and photovoltaic energy (50%), considering the latter as an environment-friendly alternative. Scenario C represented the use of photovoltaic energy as the only source for the production of devitalised moss. Finally, for Scenario D was assumed that the reactor would be located in a room with constant temperature (25°C), and therefore the refrigerated bath and the external tower would not be necessary to control temperature. In this situation, a smaller refrigerated bath would be used only for the condenser and the energy use was assumed to be 10% of the existing bath based on the relation between the water flow to the condenser and to the cooling jacket. Additionally, the energy used by the external tower could be reduced to 20% of the

current demand since it would be only used to measure conventional parameters, such as pH. Taking into account these considerations, the total energy use would be reduced by 60%. The four scenarios are

represented in Figure 5, where impacts related to Scenario A (real case) are defined as 100% and the other scenarios are calculated from the base scenario.

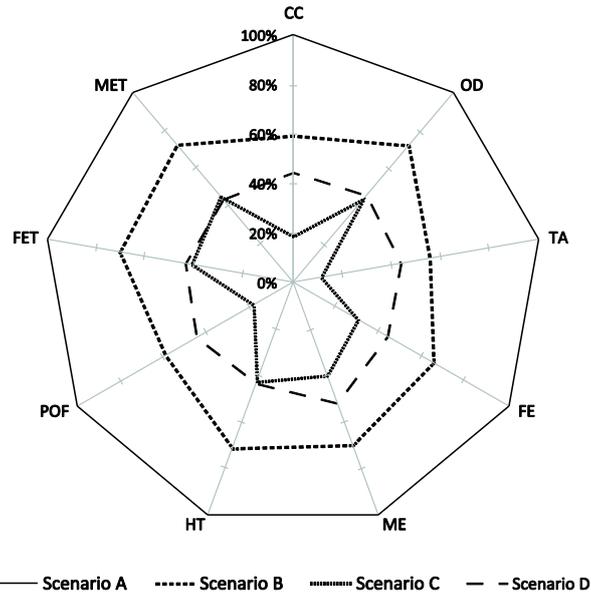


Figure 5. Comparison among scenarios proposed for moss cultivation (in %), excluding terrestrial ecotoxicity.

The outcomes showed that the environmental profile improved for all alternative scenarios assessed, except for TET, which was excluded from Figure 5 for a better display of the results. At a glance, Scenario A could be classified as the worst option, whereas Scenario C showed the lowest environmental

burdens, except for TET category (Table 3). These results are in accordance with Pérez-López et al. (2014a), who recommended the use of renewable sources to decrease the impacts associated with the use of photobioreactors.

Table 3. Results of alternative scenarios for the 10 impact categories assessed.

Impact category	Unit	Scenario A	Scenario B	Scenario C	Scenario D
CC	kg CO ₂ eq	90.44	53.69	16.93	40.25
OD	kg CFC-11 eq	7.31 · 10 ⁻⁶	5.28 · 10 ⁻⁶	3.25 · 10 ⁻⁶	3.35 · 10 ⁻⁶
TA	kg SO ₂ eq	0.65	0.36	7.51 · 10 ⁻²	0.29
FE	kg P eq	4.18 · 10 ⁻²	2.72 · 10 ⁻²	1.26 · 10 ⁻²	1.82 · 10 ⁻²
ME	kg N eq	2.24 · 10 ⁻²	1.57 · 10 ⁻²	9.03 · 10 ⁻³	1.16 · 10 ⁻²
HT	kg 1,4-DB eq	46.24	33.05	19.87	20.09
POF	kg NMVOC	0.33	0.20	6.16 · 10 ⁻²	0.15
TET	kg 1,4-DB eq	5.31 · 10 ⁻³	1.69 · 10 ⁻²	2.84 · 10 ⁻²	2.39 · 10 ⁻³
FET	kg 1,4-DB eq	0.94	0.67	0.39	0.41
MET	kg 1,4-DB eq	0.96	0.70	0.44	0.42

After Scenario A, Scenario B showed the highest impacts, presenting larger burdens than Scenarios C and D in all the evaluated categories, except for TET category. Regarding Scenario D, this option resulted to be the best option together with Scenario C for impact categories such as FET, MET, OD and HT, whereas it was the second best option for CC, TA, FE, ME and POF. When it comes to TET, Scenario D reduced the impact when compared to the baseline scenario whereas Scenarios B and C presented the largest impacts due to the use of photovoltaic energy. CC impact associated with Scenario A (90.44 kg CO₂ eq per FU) was reduced up to 81% for Scenario C. These results proved that photovoltaic energy is considerably more beneficial than the production of electricity from the grid when considering the avoided CO₂ emissions to the atmosphere. Moreover, in terms of CO₂ emissions, Scenario D with lower energy

demand showed better results (56% of reduction) than Scenario B, which combined the use of energy from the grid and photovoltaic electricity at the current use of energy (41% of reduction). Scenario A showed the highest OD impact: 7.31 · 10⁻⁶ CFC-11 eq per FU, which was reduced by ~28%, 54% and 56% for Scenarios B, D and C, respectively. The option of combining two different energy sources led to higher burdens whereas the other two alternatives showed similar results. In this case, photovoltaic energy as the only alternative source and energy reduction appeared to be the most beneficial options for the environment.

TA showed a similar profile to CC, where Scenario D was more recommendable than B, presenting reductions of 56% and 44%, respectively whereas Scenario C reduced the impact by 90%. The production of 1 kWh of photovoltaic energy led to

$2.22 \cdot 10^{-4}$ kg SO₂ eq per FU whereas 1 kWh of energy from the grid showed $2.24 \cdot 10^{-3}$ kg SO₂ eq per FU.

When it comes to FE and ME, Scenario C showed the lowest impacts, followed by D and B. If only renewable sources were used (Scenario C), environmental burdens would be reduced by 70% and 60% for FE and ME, respectively. POF outcomes were comparable to CC, TA, FE and ME profiles: Scenario C showing the best results with 82% of reduction in comparison with Scenario A, followed by D and B, with reductions around 56% and 41%, respectively. As observed in Figure 5, FET, MET and HT showed a similar profile to OD category, reductions being around 30% for Scenario B and 55–58% for Scenarios C and D. Therefore, in terms of aquatic ecotoxicity and human toxicity, the reduction of energy requirements is as suitable as the use of photovoltaic energy as the only source. Unlike the rest of impact categories, Scenarios B and C showed higher impacts than the base scenario in TET (Table 3). In fact, TET is the only category where photovoltaic energy led to much higher environmental burdens than the energy from the grid: $9.88 \cdot 10^{-5}$ vs $1.79 \cdot 10^{-5}$ kg 1,4-DCB eq per kWh produced. Although in both cases silver emissions released to air were responsible for the impact, photovoltaic energy emitted higher concentrations than energy used from the grid. On the other hand, TET was reduced by 55% when Scenario D was compared with Scenario A, with the reduction of energy as the best alternative to take in this specific case. Nevertheless, it should be noted that the damaging effect of photovoltaic energy was only observed for TET while this source presented the lowest impacts for the rest of categories, turning Scenario C into the most beneficial alternative despite the effect observed in TET.

3.3 Sensitivity analysis

The system under study was located in Spain and consequently, the Spanish energy profile was

considered for the calculations. However, noticing the relevant contribution of energy use in the total impact, the alternative of taking the energy from a different country was considered interesting for the environmental assessment. For this reason, the LCA study was performed considering the process for average electricity production in Europe and relevant differences were observed in the total impact for the assessed categories. Considering the European mix included in Ecoinvent, the process showed environmental burdens approximately 3 times higher than in the baseline scenario for FE, MET and FET with impacts per FU of 0.14 kg P eq, 2.45 kg 1,4-DCB eq and 2.42 kg 1,4-DCB eq, respectively. The reason behind this is that the typical average European mix relies more on fossil sources than the updated Spanish profile, leading to higher factors per kWh produced, such as 0.56 kg CO₂ eq instead of 0.31 kg CO₂ eq calculated for Spain. Even so, when it comes to comparing the share of each subsystem in the total impact, Subsystem 4 was again the main contributor, with similar contributions to those observed for the baseline scenario. Additionally, the importance of energy use was underlined as the main hotspot in the assessment, showing similar shares to the Spanish profile.

Regarding the improvement scenarios, the average European mix was considered for Scenarios A, B and D, whereas the single use of photovoltaic energy was considered for Scenario C. In general, the same conclusions were obtained from the analysis, where Scenario C presented the best performance, while Scenario A was the least recommendable option (Figure 6). However, it should be highlighted that the high impact associated with the European energy production led the use of photovoltaic energy (Scenario C) to perform better than Scenario D in all categories, except for TET, while using Spanish energy mix both Scenarios showed similar reductions in FET, MET, OD and HT.

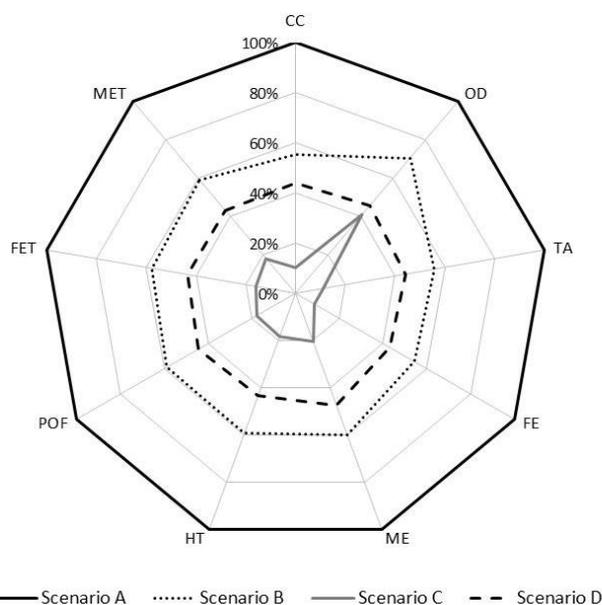


Figure 6. Comparison among scenarios (in %) using the European energy mix and excluding terrestrial ecotoxicity.

4 Conclusions

The main objective of this research paper was to perform a life cycle assessment of the production of dry moss, which is used as air quality biosensor that can accumulate atmospheric pollutants. The production process was assessed and inventoried for 1 batch of production in a 15 l glass photobioreactor. The system was divided into 5 subsystems and the key environmental burdens were identified for each subsystem and for the global process. The significant use of energy in Subsystem 4, which corresponded to the cultivation of moss in the photobioreactor, led to the highest impact in all categories evaluated. Although the reactor was continuously illuminated, the highest consumption of energy is associated to an external refrigerated bath, used for the condenser and for maintaining constant temperature. Considering this point as the hotspot of the process, improvement alternatives were suggested, based on the total energy required but also in the energy source.

Summing up the outcomes from the environmental assessment, the lowest impacts in the different impact categories were attained when actions are taken to reduce the energy consumption and only photovoltaic energy is used. Moreover, the partial implementation of photovoltaic energy may be a compromise solution with acceptable reduction of environmental impacts and easier implementation in the process.

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Naujo biotechnologinio jutiklio, pagrįsto devitalizuotų samanų klonu, vertinimas aplinkosauginio veiksmingumo požiūriu oro kokybei stebėti

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Pastaraisiais metais buvo siūloma naudoti samanas kaip galimus biomonitorius oro kokybei stebėti. Tinkamiausių tokių biojutiklių tipą sudaro samanos, užaugintos neužterštose natūraliose buveinėse arba fotobioreaktoriuose. Buvo įvertinti samanų gamybos ir jų devitalizavimo procesai, siekiant nustatyti procesų etapus, kuriuose daromas didžiausias poveikis aplinkai. Pagrindinis aspektas, kurio poveikis jūrinei eutrofikacijai sudaro nuo 85 proc., o likusioms kategorijoms – iki 95 proc., buvo energijos sąnaudos, ypač susijusios su šaldymo procesu samanų auginimo fazėje. Rezultatai buvo palyginti su ankstesnėmis studijomis, susijusiomis su dumblių gamyba fotobioreaktoriuose. Juose elektros sąnaudos apšvietimui taip pat buvo pagrindinė kritinė problema. Buvo pasiūlyti scenarijai, pagrįsti sumažėjusiu energijos poreikiu ir alternatyvių energijos šaltinių diegimu. Jie parodė geresnį aplinkosauginį veiksmingumą negu bazinis scenarijus. Visose tirtose kategorijose, išskyrus sausumos ekotoksiškumą, dėl fotovoltinės energijos naudojimo poveikis aplinkai galėtų sumažinti 50 proc., tačiau fotovoltinės energijos naudojimas kartu su tradicine energija lemtų žymiai mažesnį veiksmingumo rodiklį. Šaldymo sistemos optimizavimo galimybė taip pat lemtų reikšmingus sutaupymus ir būtų laikoma geriausia alternatyva, jei būtų tiriamas sausumos ekotoksiškumas.

Raktiniai žodžiai: oro kokybė, aplinkosauginis vertinimas, būvio ciklo vertinimas (LCA), samanų klonavimas, fotobiorektorius.