AgrEcoMed

FOSTERING AGROECOLOGICAL TRANSITION

“New AGRoecological approach for soil fertility and biodiversity restoration to improve ECOnomic and social resilience of MEDiterranean farming systems”

Deliverable 3.5 Part 1
LCI Life Cycle Inventory of Biostimulants

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PRIMA
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IN THE MEDITERRANEAN AREA

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Acronym and abbreviations

LCI Life Cycle Inventory

LCA Life Cycle Assessment

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Executive summary

Deliverable 3.5 highlights the significance of plant biostimulants in modern agriculture. These optional substances can reduce fertilizer requirements and environmental pollution while improving crop quality and yield. Biostimulants support sustainable agriculture in line with the European "Farm to Fork" strategy for an agroecological approach. They can be applied to soil or through foliar treatments. The deliverable provides an overview of the LCI/LCA methodology in ISO 14040 and 14044 standards for LCA, the SETAC LCA Handbook, and the UNEP/SETAC Life Cycle Initiative, as applied to biostimulant production. Primary data were collected from Hydrofert, Andria, Italy, on biostimulant production from lignite, Fabaceae, and seaweed. Secondary data were sourced from scientific literature and Ecoinvent and Gabi databases. The collected data were recorded using the software LCA for experts (Sphera Solutions, Chicago, USA), which will be used for subsequent LCA analysis of experimental wheat tests envisaged in the project.

Keywords:

Biostimulants, Life Cycle Inventory, Life Cycle Assessment, AgrEcoMed

1. Introduction

Due to the increasing problems of global climate change and food security, different mitigation strategies are being studied. The main goal of modern agriculture is, not only to develop sustainable systems, but also to reduce the inputs without reducing the quality and yield of agricultural systems.

Within this context, plant biostimulants are alternatives that can decrease fertilizer requirements and environmental pollution, at the same time, they increase the resistance to abiotic and biotic stresses (Irani at al., 2021), improve nutrient use efficiency and, enhance crop yield and quality (Bulgari et al., 2014): therefore, they represent a high-level sustainable strategy.

In the last European regulation 2019/1009 (Reg. EU 2019/1009) (rules on safety, quality and labeling for fertilizers are introduced and seven types of fertilizing products ("Functional product categories" FPC) have been named. Particularly, biostimulants are considered as part of the FPC6 and they are divided into microbial (bacteria, fungi) and non-microbial, organic (humic acids, hydrolyzed protein, algae, etc.) or inorganic (mineral elements, chemical compounds, etc).

The word "biostimulant" has many different definitions (Yakhin et al., 2017). According to the European Regulation on Fertilizers (EU, 2019), plant biostimulants act on plant processes, improving nutrition and vigor and they follow this definition: "A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content." Biostimulants originate from different materials, both organic and inorganic (Calvo et al., 2014); the most common components of the biostimulants are mineral elements, humic substances (HSs), vitamins, amino acids, chitin, chitosan, and poly- and oligosaccharides (Berlyn & Russo 1990; Hamza & Suggars 2001; Kauffman et al. 2007). The use of biostimulants in agriculture is increasingly frequent, as they are substances necessary to obtain abundant and healthy productions, but at the same time, it is also necessary to identify innovative sustainable formulations in line with the Farm to Fork (F2F) methodology.

Biostimulants are different materials from fertilizers since they promote plant growth even when applied in low doses. Furthermore, they increase the assimilation capacity of the nutritive elements, allowing for more abundant, quality productions and with a lower use of non-renewable resources. Many studies have addressed the important role of biostimulants in increasing crop productivity, in inducing physiological and biochemical responses in crops (enhance root development, increase photosynthetic activity in vegetative tissues, etc.). Irani et al. (2021) showed that biostimulants limit the drought stress in grapevine; Santini et al. (2021) studied the properties and the potential of cyanobacteria used as biostimulants in agriculture. Whereas Szparaga et al. (2019) evaluated the effect of the use of biostimulants on bean cultivation on biometric traits, nutraceutical quality, fiber content, and antioxidant potential.

Hamedani et al. (2020) focused on the environmental sustainability of biostimulants and evaluated the environmental impacts and the carbon footprint of mycorrhization and biostimulants of zucchini and spinach plants under greenhouse conditions. According to their results, biostimulant application enhanced spinach yield, especially under low nitrogen supply, and the nitrogen use efficiency was higher in the treatment that included the leaf supply of biostimulants compared to those with only mineral fertilization of the soil. The results obtained from the carbon footprint estimation showed that both mycorrhization and foliar applications of vegetal-derived protein hydrolysates can lead to a 7–12% and 7–

24% reduction in the global warming potential of greenhouse-grown zucchini and spinach, respectively (Hamedani et al., 2020).

The aim of this study is to evaluate an inventory (LCI) and the environmental load generated by the production of biostimulants based on vegetable-derived protein hydrolysate (LCA). The results of this study will be used during the Prima AgrEcoMed project in order to draw up an overall LCA study applied to wheat crops.

2. Materials and Methods

Life Cycle Inventory (LCI) is a relevant phase of Life Cycle Assessment (LCA), which is a comprehensive method for evaluating the environmental impacts of a product, process or service throughout its life cycle. Environmental analysis through LCA is an analytical tool that allows to measure, manage and communicate the environmental loads related to production processes and products, including greenhouse gas emissions (100-year Global Warming Potential GWP100). This tool is based on the standardized methodology (ISO 14040 and ISO 14044), through which all stages related to the process/product system are analyzed: from the production of raw materials to packaging and transport, up to end-of-life disposal.

The first step in an LCA is to describe the goal and scope of the study, which implies the definition of the system boundaries and also the functional unit to which both the inventory data and the impact results will be referred. The purpose of this analysis is to provide an environmental profile and comparison of the production of biostimulants produced from lignite, legumes, and algae. The system boundaries include the production of raw materials from agricultural crops or the appropriation of natural resources followed by the processes carried out in the company, the packaging and the waste generated necessary for the production of the finished product. The production of capital goods (i.e. buildings and machinery) is excluded from the analysis as it relates to an industrial system of long life. The functional unit to which both the inventory data and the environmental impacts will be referred is the volume unit in which biostimulants are sold, namely 1 l, 5 l, and 20 l; in this way, the influence of the packaging on the environmental profile of the biostimulants will be analised.

LCI provides a detailed inventory of all inputs and outputs associated with a product or service, from raw material extraction to disposal, to quantify its environmental impacts. The LCI process involves the collection of data on energy and material inputs, as well as emissions and waste outputs, at each stage of the product's life cycle. This data is then compiled and analyzed using specialized software to produce a comprehensive inventory of the product's environmental impacts. In the context of life cycle assessment (LCA), primary data and secondary data refer to two different types of information sources.

Primary data are typically more accurate and reliable than secondary data since they are collected specifically for the purpose of the LCA study. Primary data are data that are collected directly from the source, such as from a manufacturing facility or through field measurements , in this study data were provided from the company Hydrofert s.r.l. located in Andria (Puglia, Italy). On the other hand, secondary data are pre-existing data that have been collected for other purposes, such as from government reports or industry databases.

3. The production cycle of biostimulants

The European Green Deal aims to make Europe the first climate-neutral continent by 2050. Biostimulants are products obtained from processes that start with vegetable substances from which the active ingredients are extracted. In this analysis, the production data was obtained from the company Hydrofert s.r.l. at Andria, Puglia, Italy. The author thanks Dr. Lorenzo Vecchietti for the collaboration provided for this research.

The agrochemical and agricultural sectors play a central role in achieving this goal. These sectors can and must commit to less impactful production and use of fertilizers, which allow organic and sustainable agricultural production.

Companies in the sector are developing a large number of new products for agriculture, including biostimulants of plant or algae origin characterized by raw materials, plants, technical solutions and production processes with minimal emissions of climate-altering gases.

The company's electricity consumption has a percentage of renewable energy produced by photovoltaic panels. From the observation of company bills it was established that this percentage is equal to 7.6%..

The energy consumption for transport by train, ship and road was deduced from the processes present in the Managed LCA Content database (Sphera Solutions, Chicago, USA).

In this research on biostimulants, the inventories of three biostimulants were collected: a) lignite-based biostimulants imported into Italy from the USA (BL) Tab.1. b) biostimulants based on Fabaceae produced at 30km (BF) Tab.2. c) biostimulants based on dried seaweed imported from Ireland (BA) Tab.3.

Table 1. Lignite-based biostimulants (LB).

	production place	distance	quantity / unit of measure	process
Input				
Lignite (Leonardite)	Scraton, Nord Dakota, USA	Scraton - Houston rail 2277Km; Houston - Napoli 10769.3 km; Container ship ocean; Napoli - Andria 212 km road	840 kg	Lignite dust (Gabi DB)
Plastic bottles	Barletta, Puglia, IT	Andria-Barletta 33 km	1l 130g, 5l 330g, 20l 1080g	Plastic injection moulding (parameterized) Gabi (DB)
KOH potassium hydroxide	Canosa, Puglia, IT	Andria-Canosa 21 km	225 kg	Potassium hydroxide (Ecoinvent DB)
Water (Osmotized)	in place by tap water		2960 l	Water (deionised) (Gabi DB)
Energy (electricity)			1428,9 kW	Italian Energy mix (Gabi DB)
Output				
Organic residues	Andria, Puglia, IT		400kg; 200kg solid ; 200 kg water	treatment of biowaste, industrial composting (Ecoinvent DB)
lignite-based biostimulants (LB)	Andria, Puglia, IT		1200 kg density 1,1 kgl^{-1}	

Table 2. Fabaceae-based biostimulants (FB).

	production place	distance	quantity / unit of measure	process
Input				
Lupine and field bean residues (50%);	near Foggia, Puglia, IT	40 km	200 kg	Fava bean production, organic (Ecoinvent DB)
Plastic bottles	Barletta, Puglia, IT	Andria-Barletta 33 km	1l 130g, 5l 330g, 20l 1080g	Plastic injection moulding (parameterized) Gabi (DB)
KOH potassium hydroxide	Canosa, Puglia, IT	Andria-Canosa 21 km	225 kg	Potassium hydroxide (Ecoinvent DB)
Enzymes	Barletta, Puglia, IT	Andria-Barletta 33 km	3 kg	enzymes production (Ecoinvent DB)
Water (Osmotized)	in place by tap water		3600 l	Water (deionised) (Gabi DB)
Energy (electricity)			1429 kW	Italian Energy mix (Gabi DB)
Output				
Organic residues	Andria, Puglia, IT		800 kg; solid 350 kg + 450 kg water	treatment of biowaste, industrial composting (Ecoinvent DB)
Fabaceae-based biostimulants (FB)	Andria, Puglia, IT		1200 kg density 1,1 kgl^{-1}	

Table 3. Algae-based biostimulants (Ascophyllum nodosum) (AB).

	production place	distance	quantity / unit of measure	process
Input				
Algae dried and desalinated	Ireland, offshore	wet algae from sea to cost 5.5MJ kg^{-1}; 20km road; dried algae: from Trà Lì, Ireland to Andria Italy 2688km road	200 kg	Organic residues
Plastic bottles	Barletta, Puglia, IT	Andria-Barletta 33 km	1l 130g, 5l 330g, 20l 1080g	Plastic injection moulding (parameterized) Gabi (DB)
KOH potassium hydroxide	Canosa, Puglia, IT	Andria-Canosa 21 km	34 kg	Potassium hydroxide (Ecoinvent DB)
Water (Osmotized)	in place by tap water		3766 l	Water (deionised) (Gabi DB)
Energy (electricity)			1269 kW	Italian Energy mix (Gabi DB)
Output				
Organic residues	Andria, Puglia, IT		500 kg; solid 200 kg + 300 kg water	treatment of biowaste, industrial composting (Ecoinvent DB)
Algae-based biostimulants (AB)	Andria, Puglia, IT		1000 kg density 1,2 kgl^{-1}	

Fig.1 shows quantitatively the inputs introduced into the reactor and then into the decanter. A wet mass (400 kg) is separated by the decanter and sent to composting. All machinery used in the process consumes electricity and the energy consumed was calculated from the machinery operating hours multiplied by the relative power. The bottling is done with containers in HDPE (high density polyethylene) of the volume of 1l (weight 130g), 5l (330g), 20l (1080g). The different packaging will be the subject of scenario analysis.

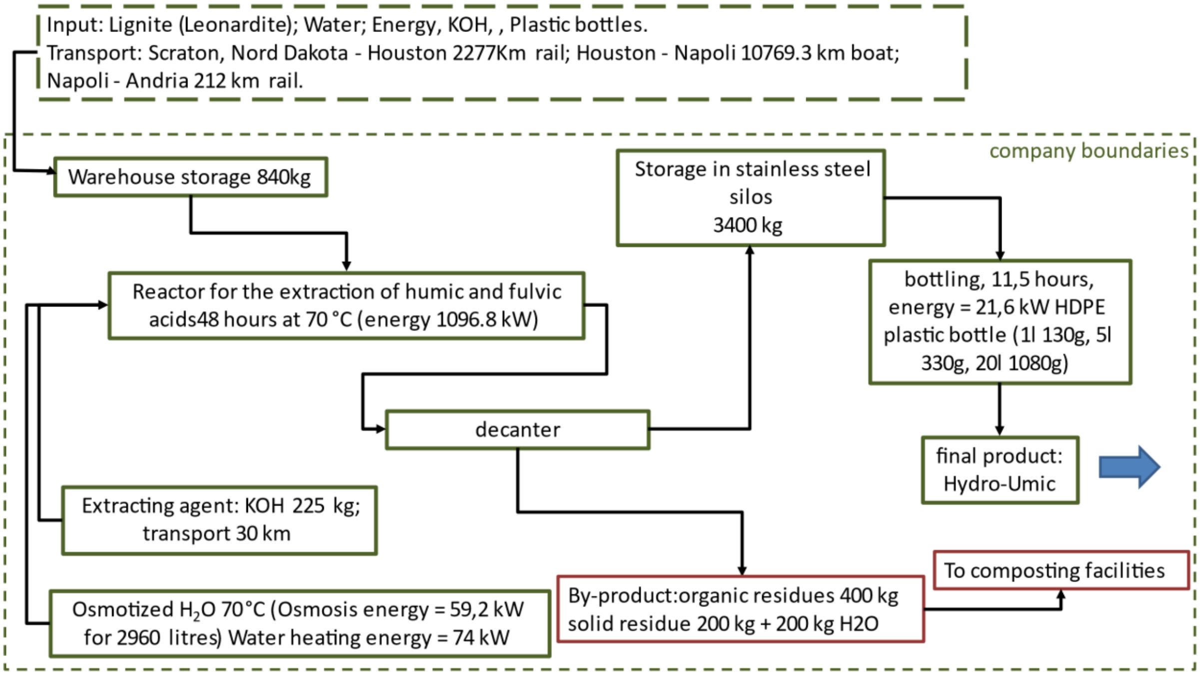


Figure 1. Production cycle of Lignite-based biostimulants BL.

Fig. 2 shows the production cycle of a biostimulant based on Fabaceae BF. Lupine and field bean residues are grown 30 km from the biostimulants manufacturer which buys only agricultural production waste. Scenario analyses will be made considering legumes as coming from agricultural cultivation or as waste from the legume supply chain. The legumes are ground into powder and introduced into the hydrolysis reactor. The effluent is pasteurized, filtered, and concentrated before being bottled. From the filtration 800 kg waste is separated, of which 350 kg of solid matter is sent to composting. All machinery consumes electricity. The company has a photovoltaic system which covers 7.6% of company consumption. The bottling is done with containers in HDPE (high density polyethylene) of the volume of 1 l (weight 130 g), 5 l (330 g), 20 l (1080 g).

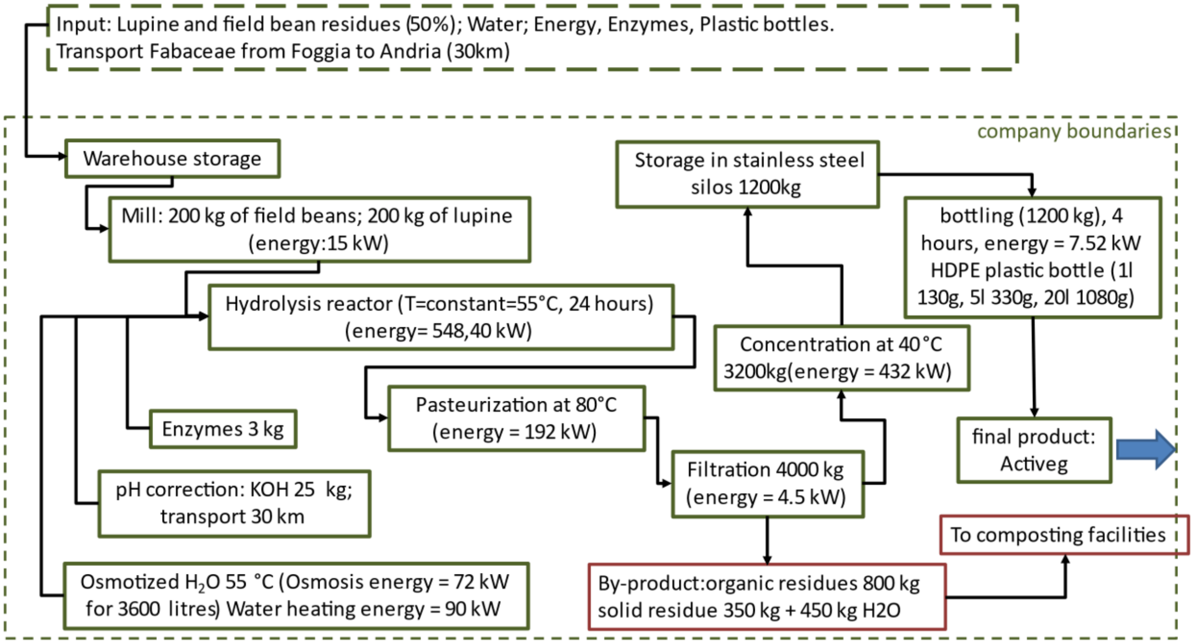


Figure 2. Production cycle of Fabaceae biostimulants BF.

Fig. 3 shows the production cycle of a biostimulant based on BA algae. The seaweed (Ascophyllum nodosum) is harvested in Ireland. The data relating to cultivation, harvesting with special boats and transport from the shore to the selling companies, and drying treatments in open swaths, were taken from the literature on algae production (Milledge & Harvey, 2016) and amount to 558.8 MJ per 100 kg of dried seaweed. As to the transport to Italy, the distance from Trà Lì on the west coast of Ireland to Andria 2688Km was considered. Once in the processing plant, the algae are introduced in the hydrolysis reactor, the effluent obtained is filtered and concentrated before bottling. From the filtration, 500 kg waste is separated, of which 200 kg of solid matter is sent for composting.

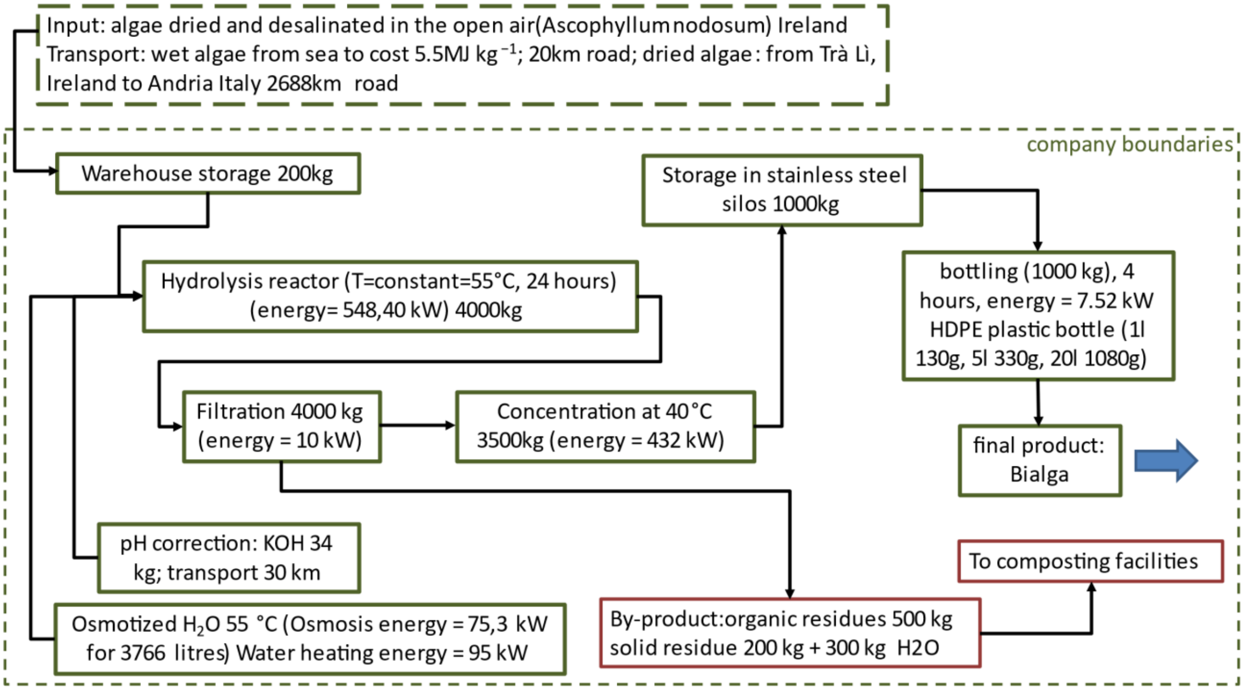
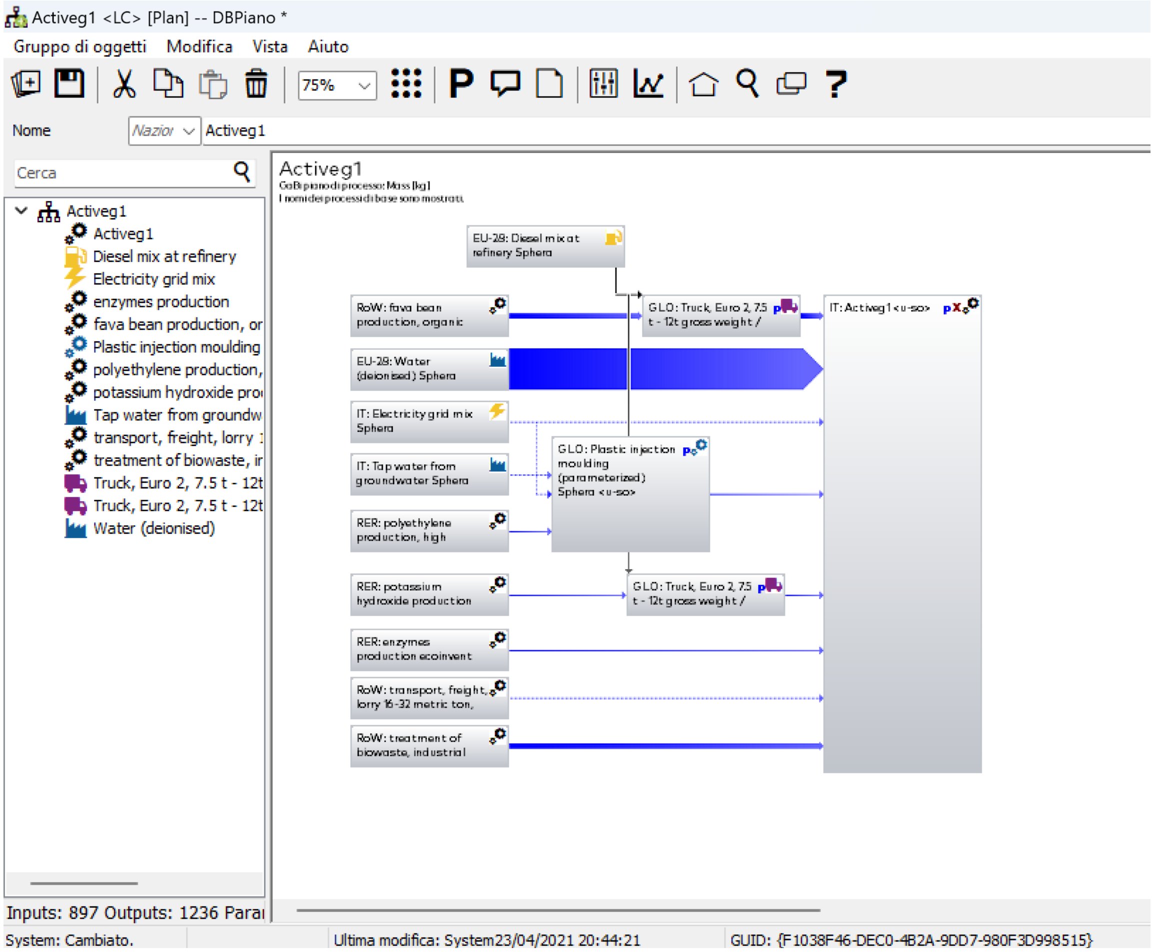


Figure 3. Production cycle of Algae biostimulants BA.

All collected data were stored in the LCA for experts software (Fig. 4). In the continuation of the AgrEcoMed. The environmental profile of the biostimulants will be assessed and integrated into the LCA of the crop rotations carried out in the project.



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System: Cambiato.

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Figure 4. Storage of inventory data in the Sphera software.

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FOSTERING AGROECOLOGICAL TRANSITION

“New AGRoecological approach for soil fertility and biodiversity restoration to improve ECOnomic and social resilience of MEDiterranean farming systems”

Deliverable 3.5 Part 2
LCI Life Cycle Inventory of Mycorrhizae

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Acronym and abbreviations

LCI Life Cycle Inventory

LCA Life Cycle Assessment

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Executive summary

Deliverable 3.5 Part 2 highlights the significance of plant mycorrhizae in modern agriculture. Mycorrhizae are beneficial fungi that establish in plant roots and have recently gained importance as biostimulants. These organisms favor the absorption of minerals and water, which translates into a reduction in the use of chemical fertilizers. In addition, they act as a protective barrier against the attack of harmful microorganisms and have a positive impact on plant stress. In this way, mycorrhizae contribute to the support of sustainable agricultural practices. This deliverable provides an overview of the LCI/LCA methodology in ISO 14040 and 14044 standards for LCA, the SETAC LCA Handbook, and the UNEP/SETAC Life Cycle Initiative, as applied to biostimulant production. Data were collected from the scientific literature, which allowed to develop four scenarios. Additionally, secondary data were sourced from Ecoinvent v3.9. The collected data were recorded using LCA for experts (Sphera Solutions, Chicago, USA)., which will be used for subsequent LCA analysis of experimental wheat tests envisaged in the project.

Keywords:

Life Cycle Inventory, Life Cycle Assessment, mycorrhizae, pot, on-farm production.

1. Introduction

Due to the increasing problems of global climate change and food security, different mitigation strategies are being studied. The main goal of modern agriculture is, not only to develop sustainable systems but also to reduce the inputs without reducing the quality and the yield of agricultural systems.
Mycorrhizae are fungi that associate with plant roots; they are fundamental in ecosystems. The use of arbuscular mycorrhizae fungi as biostimulants helps the growth and development of diverse plant species (Berruti et al., 2016), offering advantages for both the plants and the environment. This symbiotic cooperation has been evolving for years and proving vital for the success and survival of many plant species (IJdo et al., 2011). The use of mycorrhizae in horticultural crops has increased in the last two decades, mainly due to its ability to ensure production and yield stability in an environmentally sustainable manner (Rouphael et al., 2015).

Some of the benefits presented by mycorrhizae are the efficient transfer of nutrients and water between the two organisms (Jansa et al., 2003; Noceto et al., 2021); improving soil quality, and reducing the need for chemical fertilizers. Mycorrhizae form structures called hyphae, which extend into the soil and act as extensions of plant roots. These structures increase the ability of plants to absorb nutrients such as phosphorus, nitrogen, zinc, and among others (Clark & Zeto, 2000; Solaiman et al., 2014) nutrient uptake is particularly important in impoverished or degraded soils (Ryan & Graham, 2002), where plants may have difficulty accessing nutrients needed for optimal development. Mycorrhizae fungi also improve plant resistance to pests or diseases. They form a physical barrier around plant roots, preventing the entry of pathogens. Additionally, they enhance plant tolerance to drought stress, salinity, and heavy metals (Brito et al., 2019; Pozo & Azcón-Aguilar, 2007; Wu et al., 2008). These capabilities make mycorrhizae fungi a valuable resource that helps plants establish themselves and recover the necessary nutrients for growth in agricultural, deforested, or fire-affected areas.

Of the seven types of mycorrhizae documented, arbuscular mycorrhizae and ectomycorrhizae are the most abundant and widespread (Allen et al. 2003). Arbuscular mycorrhizal (AM) fungi comprise the most common mycorrhizal association and form mutualistic relationships with over 80% of all vascular plants, whereas ectomycorrhizal (ECM) fungi are also widespread in their distribution but are associated with only 3% of vascular plant families (Brundrett 2002). The production of commercial inocula of these fungi has been increasing, particularly in the last few years. However, the production of mycorrhizal inoculants is a complex process. Two major systems for AM inocula production are (1) soil-based systems and (2) soil-less techniques.

The most common conventional methods used for large-scale production of AM include cultivation in pots with sterilized substrates. Greenhouse-based pot cultivation is the cheapest way for AM propagation wherein host plants inoculated with AM fungi are cultivated in inert substrates to maintain and propagate the AM inoculum. At the end of the growing cycle, plant roots colonized with AM and/or soil-containing propagules are harvested and dried and are used as inoculum. The technique, although simpler in terms of propagation method, suffers from some limitations, such as higher risks of contamination in the inoculum, difficulty in spores harvesting, and inconsistent production. Other tested methods of AM cultivation are hydroponics and aeroponics. However, according to Kumar et al (2017), both methods require huge amounts of nutrients, either liquid (in hydroponics) or vaporized liquid (in aeroponics), which limit their industrial application.

The in vitro culture system for AM production offers pure, sterile, and bulk contamination-free propagules which otherwise is practically difficult to achieve using conventional methods (Kumar et al., 2017). The cutting edge of this technique allows several folds of increase in spore/propagule production over conventional modes of mass production in lesser time and space. Two types of in vitro systems have been developed for the production of sterile mycorrhizal inoculum (Rouphael et al. 2015): (1) AM fungi are grown and produced on transformed plant roots, popularly known as root organ culture (ROC); and (2) AM fungi are produced on autotrophic plants which are grown such that the aerial part of the plant grows outside the Petri dish (Voets et al. 2005) or grows in a sterile tube vertically connected to the Petri dish (Dupre de Boulois et al. 2006).

The production of AM fungus inoculum on the farm by cultivating in pots is an attractive alternative that can reduce costs (Douds et al., 2004). Another benefit of on-farm production is that the isolates produced can be locally adapted when the farmers' indigenous AM fungus communities are used as starter inocula, which may be more effective than introduced ones in certain situations (Sreenivasa 1992).

In summary, mycorrhizae are crucial to increase agricultural productivity and sustainability; improving nutrient uptake, promoting the efficient use of natural resources, allowing farmers to achieve healthier and more sustainable crops, reducing dependence on chemical fertilizers, and promoting environmental conservation. In recent years, many companies that produce fertilizers have marketed mixtures of mycorrhizae and nutrients that are ready to use on crops.

This study aims to develop a life cycle inventory (LCI) and assess the environmental load generated by the production of on-farm production of mycorrhizae. The results of this study will be used in the Prima AgrEcoMed project to draw up an overall LCA study applied to wheat rotation.

2. Materials and Methods

Life Cycle Assessment (LCA) is a methodology used to examine the environmental performance of a product, thus ensuring its sustainability, according to ISO 14044/40 (International Organization for Standardization, 2007; ISO, 2022). Life Cycle Inventory (LCI) is an important phase of Life Cycle Assessment (LCA), which is a comprehensive method for evaluating the environmental impacts of a product, process, or service throughout its life cycle.

The first step in an LCA is to describe the goal and scope of the study, which implies the definition of the system boundaries and also the functional unit to which both the inventory data and the impact results will be referred. Taking into account that LCA research on the production of mycorrhizae for use as biostimulants need to be developed, the main objective of this LCA is to assess and quantify the environmental impacts associated with each stage of mycorrhizae production (Fig 1). This makes it possible to identify the areas where the greatest impacts are generated and to design strategies to mitigate or minimize them.

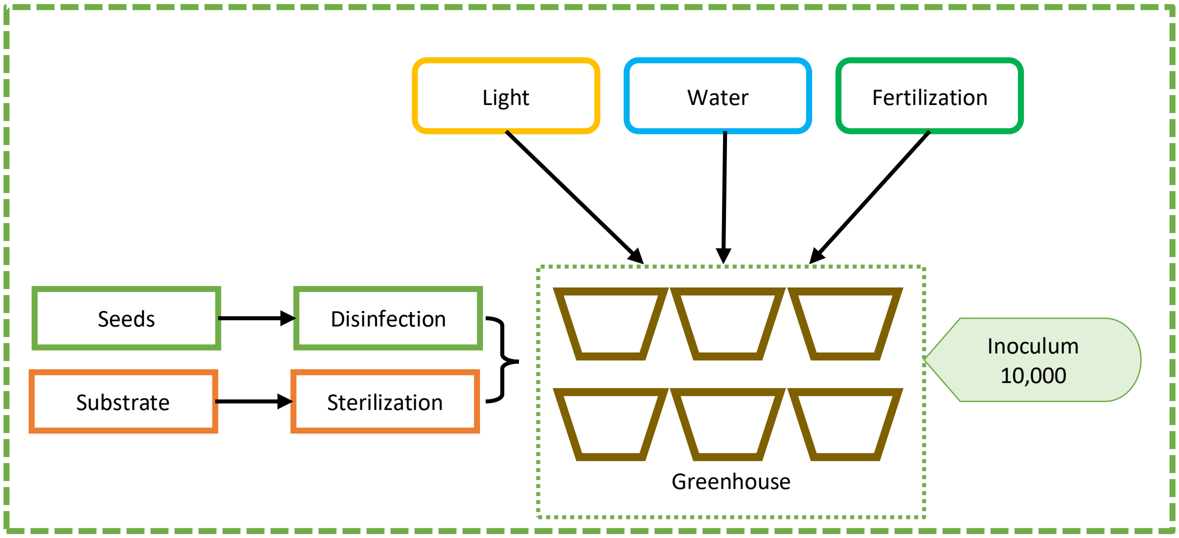


Figure 1. Production cycle of mycorrhizae.

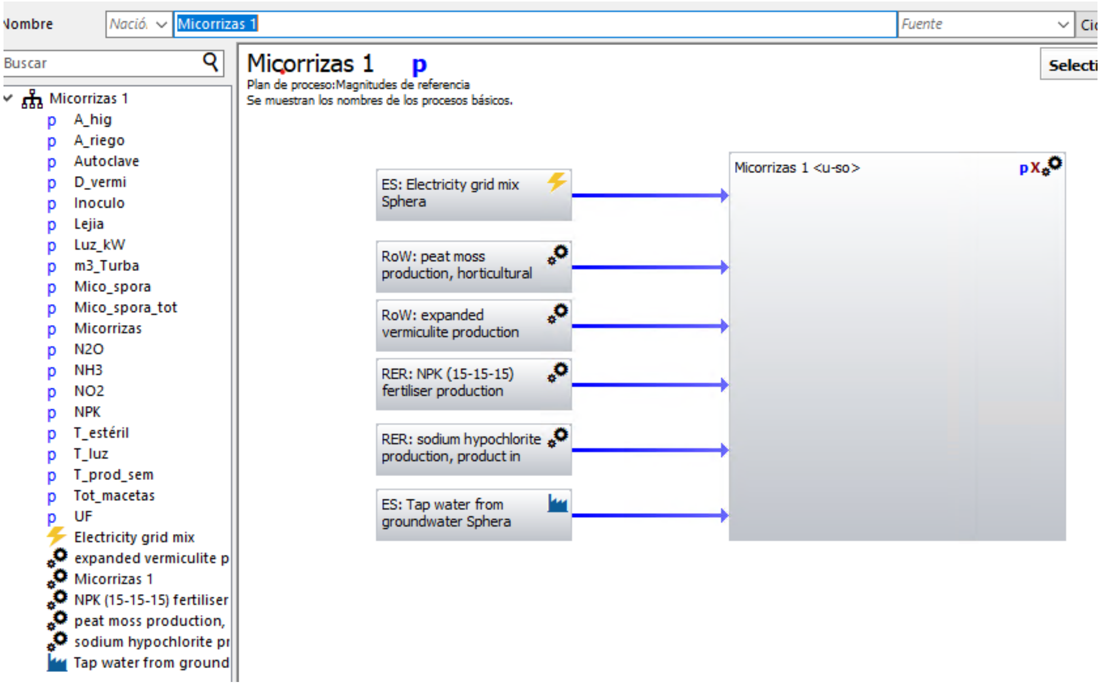
The system boundaries comprise all the processes and inputs needed to produce the inocula and comprise seed disinfection, production and sterilization of the substrate, greenhouse infrastructure, fertilizer production, and the energy needed to irrigate and light the plants. The functional unit to which all the data are referred is 10,000 spores produced per productive cycle; due to the existence of diverse species of mycorrhizae, and their structural and functional differences for their colonization, with this quantity, it would be possible to evaluate both the efficiency of the process and the consumption of resources; a higher production also indicates greater availability for their use in agricultural applications. The amount of mycorrhizae application depends on the type of crop, soil conditions, among other factors. Some recommendations are 2 kg/ha in seedbeds, or 100 to 200 gr per plant in transplanting; or the application of 1 l (10,500 spores per ml) in 4 ha of seedbeds (110,000 - 170,000 seeds) (Hijri, 2016).

Once the goal and scope of the study are defined, LCI provides a detailed inventory of all inputs and outputs associated with a product or service, from raw material extraction to disposal, to quantify its environmental impacts. The data was based on initial information obtained from the scientific literature (Miyasaka et al., 2003; Habte et al., 2001; Kadian et al., 2018; Cuenca et al., 2007), in which data were collected from laboratory experiments. Therefore four production scenarios of mycorrhizae have been developed and assessed. For each scenario, the inventory includes the amount and type of substrate used, the energy consumed for sterilization and sanitization, the water used for irrigation, as well as the fertilizer applied in each of the scenarios. In addition, on-filed emissions from fertilizers have been estimated following the IPCC (2006; 2019) guidelines. It is assumed that all scenarios would use similar equipment. For the background processes (i.e. production of fertilizers, electricity, substrate) the Ecoinvent v.3.9 database was used.

Table 1. Mycorrhizae production inventory.

		Scenario I	**Scenario II**	**Scenario III**	**Scenario IV**
Inputs	Units	(S.C. Miyasaka et al., 2003)	(Habte et al., 2001)	(Kadian et al., 2018)	(Cuenca et al., 2007)
Seeds	-	02-06	corn	10 sorghum	bean
Total time weeks	weeks	14	14	13	16
Days	Days	98	98	90	112
Disinfection	hypochlorite	5%	5%	5%	5%
Substrate /pot		(1:3) v/v*	(1:1) w*	(1:3) w*	(1:1)v/v*
Soil	kg		1	1	-
Peat moss		1	-	-	-
Silica sand	kg	-	1	1	1
Clay		-	-	-	1
Vermiculite		3	-	-	-
Inoculum	Spores	3704	1040	130	1110
Specie		Glomus aggregatum	AMF	Glomus mossae	
Energy consumption Sterilization	kWh	5,69	5	0,83	1,78
Energy LED lamp	kWh	3,57	3,13	0,488	1,28
Irrigation Water (L)	L	44,84	49,15	7,65	3,56
Fertilization	L	0,08	1,86	0,036	0,028
Emissions					
NH_3	Kg N	1,41E-07	1,13E-05	1,29E-06	4,82E-07
NO_2	Kg N	8,40E-08	6,72E-06	7,68E-07	2,88E-07
N_2O	Kg N	2,76E-06	2,21E-04	2,52E-05	9,46E-06

All collected data were stored in the software LCA for experts (Sphera Solutions, Chicago, USA) as depicted in Figures 2-5. As the AgrEcoMed project advances the environmental profile of the biostimulants will be obtained, which will be further integrated into the LCA of the rotation system carried out in the project.



Micorrizas 1

Micorrizas 1 p

Plan de proceso:Magnitudes de referencia

Se muestran los nombres de los procesos básicos.

- Micorrizas 1
  - p A_hig
  - p A_riego
  - p Autoclave
  - p D_vermi
  - p Inoculo
  - p Lejia
  - p Luz_kW
  - p m3_Turba
  - p Mico_spora
  - p Mico_spora_tot
  - p Micorrizas
  - p N2O
  - p NH3
  - p NO2
  - p NPK
  - p T_estéril
  - p T_luz
  - p T_prod_sem
  - p Tot_macetas
  - p UF
  - Electricity grid mix
  - expanded vermiculite p
  - Micorrizas 1
  - NPK (15-15-15) fertiliser
  - peat moss production,
  - sodium hypochlorite pr
  - Tap water from ground

ES: Electricity grid mix Sphera

RoW: peat moss production, horticultural

RoW: expanded vermiculite production

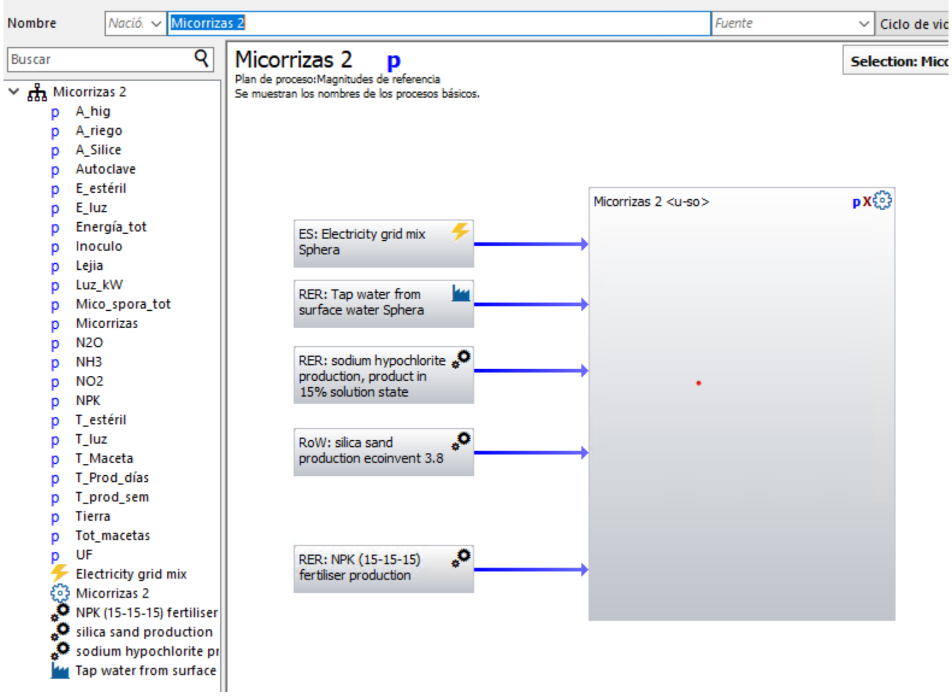
RER: NPK (15-15-15) fertiliser production

RER: sodium hypochlorite production, product in

ES: Tap water from groundwater Sphera

Micorrizas 1 <u-so> pX

Figure 2. Storage of inventory for Scenario I in software LCA for experts (Sphera Solutions, Chicago, USA).



Micorrizas 2

Micorrizas 2 p

Plan de proceso:Magnitudes de referencia

Se muestran los nombres de los procesos básicos.

- Micorrizas 2
  - p A_hig
  - p A_riego
  - p A_Silice
  - p Autoclave
  - p E_estéril
  - p E_luz
  - p Energía_tot
  - p Inoculo
  - p Lejia
  - p Luz_kW
  - p Mico_spora_tot
  - p Micorrizas
  - p N2O
  - p NH3
  - p NO2
  - p NPK
  - p T_estéril
  - p T_luz
  - p T_Maceta
  - p T_Prod_días
  - p T_prod_sem
  - p Tierra
  - p Tot_macetas
  - p UF
  - Electricity grid mix
  - Micorrizas 2
  - NPK (15-15-15) fertiliser
  - silica sand production
  - sodium hypochlorite pr
  - Tap water from surface

ES: Electricity grid mix Sphera

RER: Tap water from surface water Sphera

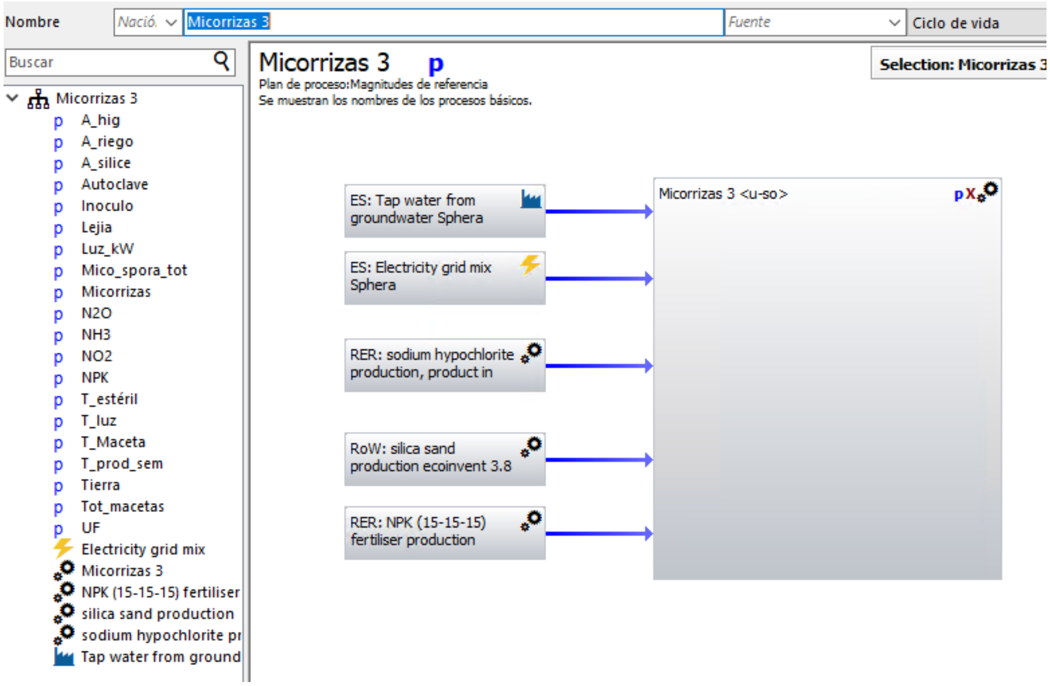
RER: sodium hypochlorite production, product in 15% solution state

RoW: silica sand production ecoinvent 3.8

RER: NPK (15-15-15) fertiliser production

Micorrizas 2 <u-so> pX

Figure 3. Storage of inventory for Scenario II in software LCA for experts (Sphera Solutions, Chicago, USA).

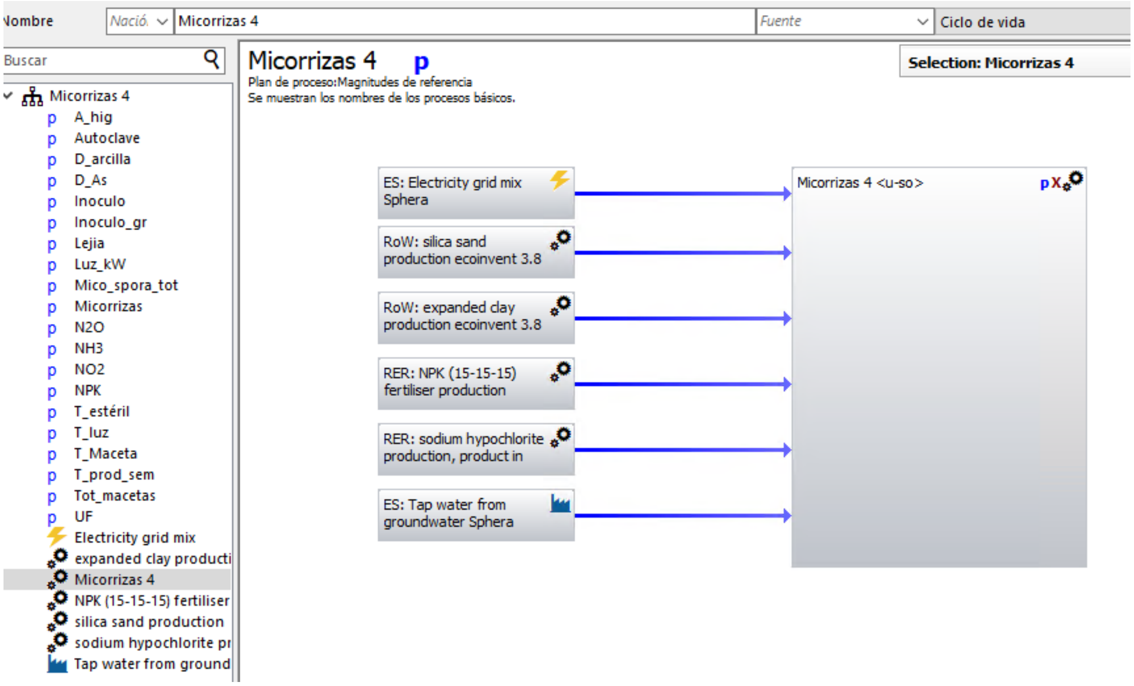

Nombre: Micorrizas 3

Micorrizas 3

Plan de proceso:Magnitudes de referencia

Se muestran los nombres de los procesos básicos.

Figure 4. Storage of inventory for Scenario III in software LCA for experts (Sphera Solutions, Chicago, USA).


Nombre: Micorrizas 4

Micorrizas 4

Plan de proceso:Magnitudes de referencia

Se muestran los nombres de los procesos básicos.

Figure 5. Storage of inventory for Scenario IV in software LCA for experts (Sphera Solutions, Chicago, USA).

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3. The production cycle of mycorrhizae

The production of mycorrhizae in pots, in general, depends on variables such as the type of plant, the substrate, or the amount of fertilizer needed. The following is a general description of the production process of mycorrhizae in pots in a non-heated greenhouse.

Production begins with the selection of seeds or plants to be associated with fungi, the seeds are disinfected with a 5% sodium hypochlorite mixture for 10 minutes and then are washed with deionized water (Kadian et al., 2018). The substrate is chosen, which can be a mixture of sand, clay, peat, vermiculite, compost, or other materials in different proportions. These are sterilized in an autoclave at 120 ºC for 60 minutes to ensure the correct infection of the fungi (Douds et al., 2004). The pots are almost ¾ filled with the substrate and the initial amount of inoculum and the seeds of the chosen plant. These are watered daily, in the presence of light for 16 hours per day, and with low phosphorus fertilization approximately weekly, this has been reported to aid colonization and spore production (Gaur & Adholeya, 2000). Growth is monitored for 14–16 weeks to separate spores or mycelia that can be used for future inoculum.

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AgrEcoMed

FOSTERING AGROECOLOGICAL TRANSITION

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AgrEcoMed

FOSTERING AGROECOLOGICAL TRANSITION

“New AGRoecological approach for soil fertility and biodiversity restoration to improve ECOnomic and social resilience of MEDiterranean farming systems”

Deliverable 3.5 Part 3
LCI Life Cycle Inventory of frass from black soldier fly

This project is part of the PRIMA Programme supported
by the European Union, having received funding from it
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	OTHER–> Software, technical diagram, etc.	☐

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	CO: Confidential, restricted under conditions set out in Model Grant Agreement;	☐
	CL: Classified, information as referred to in Commission Decision 2001/844/EC	☐

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Acronym and abbreviations

LCI Life Cycle Inventory

LCA Life Cycle Assessment

BSF Black Soldier Fly

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Executive summary

Deliverable 3.5.3 highlights the use of recycled waste organic matter as a fertilizer or amendment in modern agriculture. This decreases the use of chemical fertilizers, contributing to a circular economy, and increasing the soil organic carbon, which in turn, improves the soil characteristics and contributes to mitigating climate change. In this context, food-waste bioconversion using *Hermetia illucens* can support sustainable agriculture in line with the European "Farm to Fork" strategy for an agroecological approach. This deliverable provides an overview of the LCI/LCA methodology in ISO 14040 and 14044 standards for LCA, the SETAC LCA Handbook, and the UNEP/SETAC Life Cycle Initiative, as applied to frass production from *Hermetia illucens*. Primary data were collected from experiences at the laboratory scale carried out at UNIBAS. From this data, the process was upscaled using scientific literature and also performing mass and energy balances. Additional inventory data for the background process (e.g. energy production) were obtained from the Ecoinvent database. The collected data were recorded using the software LCA for experts (Sphera Solutions, Chicago, USA), which will be used for subsequent LCA analysis of experimental wheat tests envisaged in the project.

Keywords:

Frass, Hermetia illucens, Life Cycle Inventory, Life Cycle Assessment, AgrEcoMed

1. Introduction

Due to increasing problems of global climate change and food security, different mitigation strategies are being studied. Along these lines, the main goal of modern agriculture must be, not only to develop sustainable systems but also to reduce the inputs without reducing the quality and the yield of agricultural systems.

Nowadays, 17% of food produced globally is wasted (PNUMA, 2021), and by 2050 is expected that reach 1.5 billion tons of organic waste (Lopes et al., 2022). In the frame of a circular economy, looking for solutions to valorize food and agricultural waste as a source of other potential uses is viable and advantageous. *Hermetia illucens,* commonly known as black soldier fly (BSF), is an insect that is a good candidate to biodegrade waste from food (Salomone et al., 2017), or agri-food (Luperdi et al., 2023; Nakamura et al., 2016). The BSF can reduce manure accumulation by up to 56% (Sheppard, 1983), and, at the same time can generate animal protein. Taking into account that the demand for animal protein is expected to increase to 70% by 2050 (Oonincx & de Boer, 2012), the use of insects like BSF can be used to feed other animals and even humans.

The rearing of BSF generates frass as an additional output; the frass is the combination of insect feces, substratum, and feed scraps, and it means that the valuation of frass is gaining more economic and ecological importance (Gärttling & Schulz, 2022). The use of frass as a fertilizer or amendment is a promising revalorization treatment (Schmitt & de Vries, 2020), that increases the circularity of the production systems (Cadinu et al., 2020), given that the larvae can be fed with crop waste. However, the use of frass as fertilizer or amendment presents some limitations, given that the frass is not a uniform product, as the composition is affected by the feed substrates (Klammsteiner et al., 2020).

The selection of this technique instead of composting or anaerobic digestion seems to have some advantages for the management of food waste. Ferronato et al (2023) point out the following ones: reduction of nutrient leakage and spread of odors, thanks to short processing time, inactivation of pathogens, and decrease of bacterial activity. In addition, from BSFL and pupae, it is possible to obtain proteins and lipids (Cappellozza et al. 2019; Spinelli et al. 2019), which can have different uses such as feed formulation and biofuel production.

This study aims to evaluate an inventory (LCI) and the environmental load generated by the production of frass of Hermetia illucens (BSF) fed with organic waste from agriculture. The results of this study will be used during the Prima AGRO.ECO.MED project in order to draw up an overall LCA study applied to wheat crops.

2. Materials and Methods

Life Cycle Assessment (LCA) is a methodology used to examine the environmental performance of a product, thus ensuring its sustainability, according to ISO 14044/40 (International Organization for Standardization, 2007; ISO, 2022). Life Cycle Inventory (LCI) is an essential phase of Life Cycle Assessment (LCA), which is a comprehensive method for evaluating the environmental impacts of a product, process, or service throughout its life cycle.

The first step in an LCA is to describe the goal and scope of the study, which implies the definition of the system boundaries and also the functional unit to which both the inventory data and the impact results will be referred. This study aims to develop an LCI of the frass of *Hermetia illucens* to be applied as an agricultural amendment, where organic waste from agriculture is used as feed for the larvae.

The system boundaries span from "cradle to farm gate" *(Fig. 1)* and comprise all the inputs (water, energy for heating and lighting) and outputs (frass) to the environment. The production of organic waste used as food for the larvae is not included in the impact calculations since the organic waste is expected to come from the same production unit and has zero cost. In addition, the production of capital goods (machinery and greenhouse structure) has been excluded from the system boundaries as they have a long life and will be used for many years. The functional unit (FU) of the study is 1 kg of frass of *H.* illucens. Taking into account that frass is the core of the project and the BSF production is a co-product, to split the environmental loads between both co-products, a mass allocation is set.

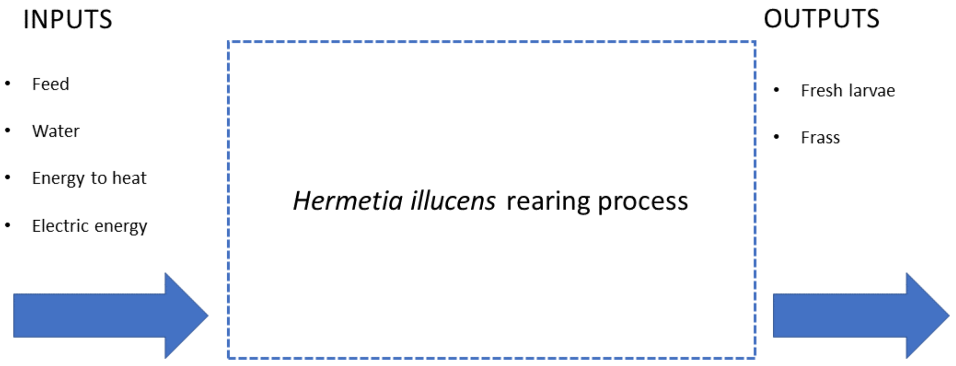

Figure 1. System boundaries of H. illucens rearing process, from "cradle to farm gate".

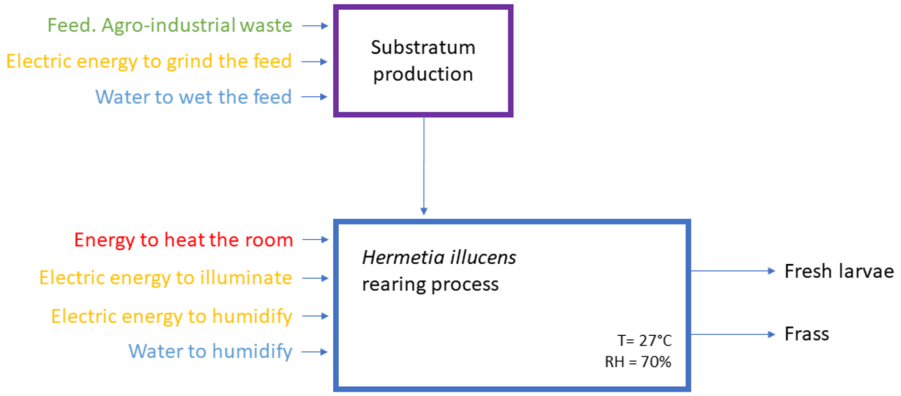

Figure 2. Process system to generate frass and BSF.

Once the goal and scope of the study are defined, LCI provides a detailed inventory of all inputs and outputs associated with a product or service, from raw material extraction to disposal, to quantify its environmental impacts. To develop the LCI (Table 1), both primary and secondary sources were used. In particular, primary data has been used for the foreground system. These data comprise the amount of feed (i.e., organic waste), water to wet the feed, fresh larvae yield, and frass yield, and come from experiments at the laboratory scale that has been upscaled. As to the background data, they have been obtained or estimated from the literature. In addition, processes concerning electricity production, water production, and natural gas production have been retrieved from Ecoinvent v3.9.

Table 1. Data inventory of the *H. illucens* rearing process.

Data	Symbol	Amount	Unit
Functional unit	FU	1	kg
Feed for the larvae	Feed	7	kg
Dry larvae	DL	2	kg
Larvae	L	6	kg
Water to wet the feed	Ww	0,70	kg
Water to humidify the environment	Wh	0,69	kg
Heating electricity	Eh	0,02	MJ
Electricity feed grinding	Eg	0,01	MJ
Electricity lighting	Ei	0,06	MJ
Nitrogen content frass	N	1,49	% w.b.
Phosphorus content frass	P	0,98	% w.b.
Potassium content frass	K	1,03	% w.b.
Cubic meter to rear H. illucens larvae kg	Vy	4,69	m^3

All collected data were stored in the software. As the AgrEcoMed project advances, the environmental profile of the biostimulants will be obtained, which will be further integrated into the LCA of the rotation system carried out in the project.

3. The production cycle of frass from *Hermetia illucens*

This system starts with conditioning the substratum, which implies grinding and increasing its water content to 70%. Therefore, the inputs of this process are agro-industrial waste as feed, electricity, and water. The rearing camera of the BSF larvae must be at 27°C and have a relative humidity of 70%. The rearing process requires as input the previously conditioned substratum, energy to heat the room, electricity to light the room for 13 hours per day, and water and electricity for the room humidification. As output, the process generates fresh larvae and the desired product, frass. The phytonutrient content of the frass is 1.49% N, 0.98% P, and 1.03% K.

The process followed to upscale the process is described below. Starting from the FU, that is, 1 kg of frass, and according to the lab scale process, the amount of feed needed is seven times that of the frass, which yields 6 kg of fresh larvae. The amount of water to be added to the feed varies depending on the waste used; however, according to our laboratory data, on average, the water needs are 10% of the weight of the feed. The energy to grind the feed has been taken from Piccino et al (2016), which suggests using the value of 16 kWh ton^{-1} to grind as a conservative approximation. Then, based on Caruso et al. (2014), the volume (m^3) needed to rear the BSF so as to obtain 1 kg of frass is calculated. From this information and applying respective mass and energy balances, the water to humidify the environment and the energy to heat the room have been estimated. The electric energy to light the room has been calculated considering a LED lightbulb of 9 W and the lighting time per day, that is, 13 hours.

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