Life-cycle assessment of a microalgae-based fungicide under a biorefinery approach

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Abstract

The aim of this work was to perform a life-cycle analysis of the production process of a fungicide based on amphidinols. Two scenarios were evaluated: (1) biorefinery process -biofungicide, fatty acids and carotenoids were considered as co-products-, and (2) biofungicide as only product. Inventory data were taken and scaled-up from previous work on pilot-scale reactors, as well as lab-scale downstream equipment. A yearly production of 22,000 L of fungicide, was selected as the production objective. Despite, photosynthetic biomass is a sink of anthropogenic CO_2, harvesting and downstream processing have large carbon footprints that exceed the biomass fixed carbon. Producing the biofungicide resulted in 34.61 and 271.33 ton of CO_{2e} (15 years) for the Scenarios 1 and 2, respectively. Different commercial agricultural fungicides were compared with the microalgal fungicide. A lower impact of the microalgal product for most of the indicators, including carbon footprint, was shown.

Keywords: life-cycle analysis, microalgae, bioprocess, fungicide

1. Introduction

The production of food of agricultural origin requires systems that resist the attack of insects, fungi, or other pests. In developed countries, national authorities are progressively reducing the number of authorized mineral chemical compounds to face pests and plant diseases. For example, European Union (EU) is constantly restricting the number of authorized chemical phytosanitary substances (at present 128 available) and is introducing more and more substances of biological origin (at present 25) (Commission Implementing Regulation (EU) 2019/716 of 30 April 2019). These are more often considered as low risk substances (Commission Regulation (EU) 2017/1432). Integrated pest management would avoid or greatly reduce the use of mineral chemicals. This strategy relies on a combination of techniques such as biological control, habitat manipulation, or use of resistant strains. This is the current standard in certain locations where the advantages have been demonstrated (i.e., greenhouse cultivation in South-East Spain). Despite that, the use of pesticides continues to be the most widely used strategy worldwide (Baker et al., 2020). Moreover, in the case of fungal pests, the use of mineral chemical or bio-sourced substances is mandatory. Although the effectiveness of these products is high, their negative impacts on the environment are also remarkable, especially in the case of pesticides without a biological origin (García Cruz et al., 2022).

Different fungicides have been used for hundreds or even thousands of years, most being simple formulations or even one-ingredient products. More recently, synthetic organic compounds have been applied (Zhao et al., 2020). Among them, there are products based on heavy metals, organophosphorus compounds, halogenated hydrocarbons, etc. Organophosphorus compounds are dangerous in the short term (Shukla et al., 2017). Its incorrect handling is associated with many acute intoxications in humans. For instance, its continued use triggers the cholinergic syndrome and is associated with multiple chronic complications, being delayed neuropathy one of the most common (Ganie et al., 2022). On the other hand, the use of non-selective fungicides based on heavy metals poses a great environmental risk, due to their known ability to bioaccumulate and their impact on other non-target species (Tchounwou et al., 2012). In recent decades, special attention has been paid to the search for alternative products, preferably bio-sourced, with less impact on the environment and on human health.

Microalgae have been proposed for several applications. For food/feed products, their advantage is their higher efficiency per unit area than that of agricultural crops (Aransiola et al., 2014). Also, microalgae are primary producers of valuable polyunsaturated fatty acids (PUFAs), antioxidant pigments and high-value metabolites with applications in nutrition, pharmacy, cosmetic industry, and others (Ketzer et al., 2018; Mehariya et al., 2021). Microalgal-based bioprocesses have a low environmental impact, including a lower carbon footprint (Chowdhury et al., 2012; Ketzer et al., 2018). Microalgal growth can be considered a CO_2, Nitrogen and Phosphate sink. (Chandra et al., 2018) or nutrients from wastewaters (Li et al., 2022). A phototrophic production process of a biofungicide would contribute to several sustainable development goals (SDGs), (e.g., Goals 9, 12 and 13) of the 2030 Agenda for Sustainable Development, adopted by all United Nations Member States in 2015.

Among microalgae, dinoflagellates stand out for being an impressive source of new bioactives with pharmacological and agro-industrial interest (Assunção et al., 2017; García Camacho et al., 2007). However, their intensive culture must cope with a number of difficulties (Gallardo Rodríguez et al., 2012; Assunção et al., 2017). These problems have been tackled in recent years through the systematic application of bioprocess engineering methodologies (García Camacho et al. 2011; López Rosales et al., 2018; Molina Miras et al., 2018). Among the bioactives-producer strains with potential for mass cultivation, the dinoflagellate *Amphidinium carteae* is one of the most promising (Molina-Miras et al., 2018; Fuentes-Grünewald et al., 2016). *A. carterae* can produce a series of secondary polyketide metabolites (López Rodríguez et al., 2019) among which are amphidinols. Amphidinols elicit antitumor, antifungal, and hemolytic bioactivities (Satake et al., 1991, Abreu et al., 2019). Their ability to interact with membrane sterols and permeabilize membranes by forming pores has allowed to patent the use of *Amphidium* extract for the production of a biofungicide (De La Crouee and Yann, 2017).

Although microalgal bioprocesses have apparently less environmental impact than chemical processes, for their proposal to replace technologies and products on the market, it is necessary to carry out rigorous studies of their environmental footprint (Reijnders, 2020). As is known, there are different methodologies for this, among which the Life-Cycle Assessment (LCA) is the one with the greatest analytical value. Besides, it can be integrated in the development of products, processes, and services through environmental design (Reijnders, 2020).

In this work, a process for manufacturing a microalgae-based agricultural fungicide is proposed. The feasibility of the process in terms of environmental impacts associated with all the stages of its life-cycle is assessed.

2. Materials and methods

The Figure 1 summarized the followed methodology. SuperPro Designer v.7 (Intelligent USA) software was used for the process design step. The software was able to provide estimates of consumptions of energy, water and other services for the non-specific equipment. Different alternatives of production including a debottlenecking process were evaluated. The objective was to avoid underused equipment and to perform a sizing optimization.

2.1. Goal and Scope definition

A LCA study of the production of a microalgal bio-based fungicide was carried out using Air.e LCA v3. 12.0.10 software (Solid Forest, Madrid, Spain). The production of the biofungicide was investigated under two process scenarios: (1) under a biorefinery approach considering two valuable co-products (fatty acids and carotenoids) (Figure 2A); (2) under a single-product process (Figure 2B). The analyzed process chain relates to a hypothetical system that is based on extrapolation from laboratory and pilot-scale experiments (described in the next section). The system boundaries for the process were established to encompass all the essential processes that are directly utilized in the production. Foreground system was separated into several sections as can be seen in Figure 2. 1 L of fungicide was taken as the functional unit.

2.2. Life cycle inventory (LCI)

The inventory data for the LCA collected below includes mineral chemical products (such as nutrients, solvents, washing agents, etc.) and electrical consumption for a production unit of the fungicide. Inventory was included in Table 1, where all the collected data have been calculated using the material and energy balances made in SuperPro Designer. As was indicated, all the data referring to the environmental impacts of the different mineral chemical products used, from cradle to door, including therefore transport, have been taken from the Ecoinvent database (v.3.7.1). There is no entry for vitamins in Ecoinvent, so we estimate the values for biotin, thiamine and cobalamin assimilating them to those of vitamin D3 (Morales González et al., 2019). Pure CO_2 has been considered because the effect of the impurities of flue gases in *A. carterae* is still unknown. Technical specifications related to the RW-PBR and other microalgal-specific equipment and processes were provided from previous publications where laboratory and pilot scale results were reported (Molina-Miras et al., 2018, Morales-Amador, et al., 2018, López-Rodríguez et al., 2019, 2020, 2021).

The following assumptions were adopted. (i) Nutrients of the culture medium were based on commercial agricultural fertilizers. No differences have been observed between lab-scale cultures grown with control chemically defined medium and that equivalent based on fertilizers (unpublished data). Nutrients were completely consumed by recycling the exhausted culture medium (Molina-Miras et al., 2020). (ii) Electricity supply came from the Spanish energy mix. (iv) The production facility was located near the Mediterranean coast, so seawater was available through pumping. (v) The process of applying the fungicide in their final destination, that is, in crops, was not considered because it is similar for liquid fungicides.

2.2.1. Microalga and fungicide

The marine dinoflagellate microalga *A. carterae* BMCC33 (strain named as Dn241EHU in previous publications), which produces amphidinol A and amphidinol B (Abreu et al., 2019), was chosen for this study. The percentage of amphidinols in its biomass is dependent on the culture conditions. Thus, a percentage of up to 0.69 % on dry weight of biomass has been reported (López-

Rodríguez et al., 2021). The minimum concentration of amphidinols present in the formulation that provides an effective fungicidal activity (inhibition of 100 %) was 64 mg L^{-1} (own determination on *Fusarium melonis*). The proposed formulation for sale to farmers should be 200 times more concentrated, this is, 12.8 g L^{-1}. Depending on the type of crop, the application rate of commercial liquid insecticides and fungicides generally varies between 1.5 and 2.5 L ha^{-1}. A 22 m^3 volume of concentrated fungicide would be approximately needed to treat an agricultural area between 8,000 and 14,000 ha per year.

2.2.2. Process description

Considering a conservative concentration of amphidinols in the biomass of 0.2% (dry weight), 4400 m^3 of *A. carterae* culture are needed to produce 22 m^3 of concentrated fungicide. A total of 12 raceway PBRs of 10 m^3 each were chosen after studying alternatives for optimizing the use of downstream equipment (see supplementary materials). This number of photobioreactors (PRBs) operated in semicontinuous mode was expected to provide around 245 annual batches. For the step of cultivation of *A. carterae* in the photobioreactor, the culture medium was prepared using Mediterranean seawater pumped from a well and filtered with a membrane filter of 0.25 µm pore. The composition of the culture medium is given by that of the f/2 medium recipe multiplied by 3 (López Rosales, et al., 2018). Concentrated medium stocks were dissolved in deionized water. The filtered seawater was sterilized in situ by chlorination (López Rosales et al., 2018). For this, 3 mL of a commercial bleach solution (4.7% of active chlorine) were added for each liter of seawater. After mixing for 5 hours in the PBR, seawater was neutralized adding concentrated solution of sodium thiosulfate (250 g L^{-1}) at a ratio of 1 mL per 4 mL of bleach. Next, nutrient stocks and vitamins were added to the seawater at the required concentrations. Then *A. carterae* seed was added; the inoculum volume being a 10% (v/v) of the whole PBR culture volume. During the period of each batch (12 days), air was supplied continuously at 0.05 vvm and CO_2 was supplied on demand with pH control (pH = 8.3) (Molina-Miras, et al., 2018). Microalgal biomass was harvested and treated every 12 days (Fig. 2A). The broth was firstly pre-concentrated by means of a lamellar settler for 2 hours. The diluted sludge obtained was then pelleted by continuous centrifugation, removing around 85% of the water. Before discharge, the biomass pellet was subjected to a washing step with deionized water to remove salts. Next, the biomass was spray-dried. In saponification step, a volume of tricomponent solvent mixture (87.5/6.5/6 of 96º ethanol, hexane, and water ratio, respectively) was added to the dried biomass at a ratio of 40:1 (v/w), together with 40% w/w of KOH, relative to dry biomass. Saponification reaction was carried out at 60ºC for 30 minutes. The crude soap from the saponification reaction and resulting solid residue were then separated by filtration. The solid residue was extracted once more with an additional volume of fresh tricomponent solvent mixture at a 16:1 (v/w) ratio (López-Rodríguez et al., 2021). The whole crude soap obtained from the saponification reaction consisting of two immiscible filtered liquid phases, one hexane and the other hydroalcoholic, was subjected to a carotenoid-oriented extraction step:

i) Extraction of carotenoids: The resulting liquid phase was introduced into an evaporator. The pellet (the mixture resulting from evaporation) was resuspended with hexane, at a 4:1 v:v ratio. It was stirred for 1 hours. Once decanted, the hexane phase undergoes evaporation, from which we obtain a fraction of apolar carotenoids, as well as recover part of the hexane used. The pellet was again subjected to solid-liquid extraction, this time using 2.5 kg of 99:1 acetone water solution, thus extracting the remaining carotenoids (polar carotenoids). It was stirred again for 5 minutes (López-Rodríguez et al., 2021). After decantation, the light fraction was evaporated, obtaining more carotenoids and recovering the solvents. Both currents of carotenoids were then mixed in a stirred tank, where they were stabilized with olive oil. Final carotenoids percentage was 2%.

ii) Extraction of saponifiable lipids (free fatty acids): The heavy phase from the previous stage was fed to a stirred tank, where we will add a mixture of water-ethanol (72.8% ethanol) in a ratio of 10:1 (v/p dry biomass). It was then mixed with hydrochloric acid to hydrolyze the previously formed soaps. For lipid extraction, hexane was added in a simple liquid/liquid extraction at a ratio of 10:1 (v/w dry biomass). The resulting mixture was introduced into a decanter. The light phase, rich in hexane, was subjected to evaporation, recovering the lipids. The heavy phase passed to a distillation stage, where the ethanol was recovered. The bottom of the distillation passed to the stage of formulating the fungicide.

iii) Fungicide formulation: The heavy phase obtained in the vacuum distillation of the previous stage iv contained amphidinols (0.049%). The fungicide was effective at 0.0085% (dilution was made with water). Extract amphidinol content was 0.426 mg/L. This concentration was for the applied product (already diluted). Thus, commercial product was 200 times more concentrated.

Scenario 2: The fungicide production process without a biorefinery is simpler. The first stages of the process are maintained, modifying the process after spray-drying with the following operations:

iv) Extraction of active fungicidal fraction: After drying, the biomass was suspended in methanol, at a rate of 20 mL/g of biomass, and at 60ºC (the bioactivity was kept until 90ºC (De La Crouee & Thiebeauld, 2017). After this, the methanolic solution was subjected to distillation for the recovery of methanol.

v) Formulation of amphidinols: the extract rich in amphidinols from the previous stage was finally dissolved in water until it reaches the required concentration.

2.3. Life cycle impact assessment (LCIA)

Allocation procedure was required since Scenario 1 involved the production of different compounds. According to ISO 14040/44, allocation was avoided by considering sub-processes that only contributed to one product. For indivisible subprocesses, the environmental burdens were allocated between the co-products on a mass-based allocation approach. The following methodologies were chosen: PAS 2050, ILCD and ISO 14046. The ReCiPe midpoint method was used for the impact assessment. The database for Air.e was Ecoinvent (v3.7.1; www.ecoinvent.org). Calculations from SuperPro Designer software for the processes design were used for the LCA.

2.4. Interpretation

The LCA findings were presented based on the principles and recommendations outlined in the ISO standards. For interpretation purposes, results were compared with those obtained for several commercial fungicides considering only their composition (Revycare®, Priaxor®, Delan® Pro, Cabrio® WG, Sercadis® and Serifel®, all of them from the German manufacturer, BASF). Due to the reliance on background Ecoinvent uncertainties and models in databases, an uncertainty analysis was carried out. It involved running 1000 simulations using the "Monte Carlo analysis" method. This approach utilized a pedigree matrix to assess the uncertainty across various midpoint impact categories

(Ciroth et al., 2016) (see supplementary materials). Outcomes of this analysis included the calculated mean value and standard deviation (SD). Unpaired t test with Welch's correction were conducted to compare the results.

3. Results and Discussion

Although in recent years there has been an increase in the number of LCAs carried out for microalgal processes, these are still scarce and, in some cases, unreliable, given the enormous variability of results and even their inconsistency (Reijnders, 2020). Furthermore, most LCAs found in the literature are focused on microalgal biodiesel production (Bradley et al., 2015; Ketzer et al., 2018; Li et al., 2022; Reijnders, 2020; Silva et al., 2015; van Boxtel et al., 2015). Notwithstanding, a few of them have been focused on high value microalgal products (Mehariya et al., 2021; Porcelli et al., 2020; Reijnders, 2020), for example feed for aquaculture, proteins, fatty acids, phycocyanin, etc.

Environmental impacts can be classified as intermediate (direct effect) or final (cumulative impacts over the entire life cycle), although the difficulty in estimating the long-term effect of emissions on the environment makes more common the use of intermediate impact methods. The ReCiPe method was used in this work due to its popularity in bioprocesses, including microalgae-based ones. For agriculture fungicides, based on the results obtained from the ReCiPe MidPoint method, the impacts of greatest interest were: Marine Water Ecotoxicity (MET), Human Toxicity (HT), Global Warming Potential (GWP), Water Resource Depletion (WD), Mineral Resource Depletion (MD), and Fossil Resource Depletion (FD). The diagram of the amphidinol production process for Scenarios 1 and 2 are identical up to the spray-dryer stage, from which stage 2 is simpler since a single product is obtained using fewer steps (see Figure 2 and supplementary materials).

The total impacts, expressed in mass or volume of equivalent resources, for the proposed process were included in Figure 3. In this Figure 3 we can see that the production of the fungicide and the co-products, under a biorefinery approach, generates higher impact per functional unit than the single-product process. This is the obvious result of producing 3 co-products. Error bars in Figure 3 are standard deviations obtained from the uncertainty analysis. Significant differences were obtained for all the impacts ($p < 0.001$). In the Figure 4, the contribution of the different stages to the impact indicators have been plotted. For Scenario 1, Carotenoids Extraction and, to a lesser extent, the Saponifiable Lipids Extraction stage were the most contributing stages. For Scenario 2, Inoculation and Culturing caused most of the impacts. Since the biomass production is the same for both Scenarios, it can be concluded that the inputs required for lipids and carotenoids were the greatest source of the environmental impacts. Similar results were obtained for other bioprocesses (Adesanya et al., 2014; Ishizaki et al., 2020; Pérez-López et al., 2017; Porcelli et al., 2020).

3.1. Global Warming Potential (GWP)

One of the most studied impact indicators in LCA, for any type of process or service, is the GWP100. This indicator is used to characterize the impact of climate change. The main GHG is CO_2, whose emissions (along with those of other GHGs and the disposal of by-products and waste products) are used to calculate the carbon footprint of a process (Mata et al., 2018). In processes in which microalgae are used, it is necessary to provide CO_2 for photosynthesis, generating biomass with this reaction. The concentration of CO_2 in the atmosphere, although increasing, is approximately 0.04 ppm, which is insufficient for an optimized process. For this reason, the injection of pure CO_2 gas is common. It is important to consider that if we keep the culture pH above 8, CO_2 losses can decrease (Collet et al., 2014), although the optimum pH for cultivation is species-specific. The consumption of CO_2 during the cultivation stage, in biomass production, can make the net production of this gas negative, although it largely depends on the efficiency of the process to really act as a carbon sink (Mata et al., 2018). The CO_2 used in our study has not been distillated from air but was produced by a chemical process. Therefore, this also influences the depletion of mineral resources. Although the use of CO_2 from a combustion stream could be a potential alternative, pure CO_2 has been considered because the effect of the impurities of flue gases in *A. carterae* is still unknown.

Electrical consumption has a very important relevance in the operating costs of microalgal bioprocesses (Acién Fernández et al., 2017). It also has a great environmental impact (they directly affect the carbon footprint and GWP) (Pérez-López et al., 2014). Pérez-López et al. estimated a consumption of 1.42 kWh per kg of microalgae during the cultivation stage based on real industrial-scale data. In our study, the calculated consumption was 1.40 kWh per kg of biomass (based on estimations from real pilot-scale data). If we consider the entire process, from the sterilization stages to the end of the downstream, the consumption measured by Pérez-López et al. (2014) was 30.45 kWh. Our electrical demand was 29.67 kWh. Therefore, the methodology followed seems correct. In most of the studies carried out with microalgae cultures, the upstream section is the one with the greatest impact on the GWP100 (Adesanya et al., 2014; Ishizaki et al., 2020; Pérez López et al., 2017; Porcelli et al., 2020). This is due to the energy consumption invested in the mixing, filtration, CO_2 supply and, in the harvesting, (for example, centrifugation). Therefore, even using PBRs with low energy consumption, the stage that most contributed to climate change was also inoculation and cultivation (Scenario 1).

The production of the culture medium ingredients (although they were fertilizers rather than technical grade reagents) is an important impact contributor since mining, chemical processes and transport are energy-intensive activities (Mata et al., 2018). One of the solutions proposed to reduce the demand for fertilizers is the use of wastewater. Diniz et al. (2017) evaluated the environmental advantages of using wastewaters, concluding that it depended largely on the required pretreatments. Besides, their use is restricted to applications non-related to direct human consumption (food or health).

In this work, electrical consumption was comparatively lower than in other studies, given the use of raceway PBRs (0.002 kW/m^3) (Acién Fernández et al., 2017; Chandra et al., 2018), as well as lamellar settlers to pre-concentrate the biomass. Thus, the use of a combination of different technologies can significantly help reduce this impact. Adesanya et al. (2014) exemplified this by implementing a hybrid culture system, with open and closed photobioreactors, achieving 42% reduction in global warming potential.

3.2. Marine water ecotoxicity (MET) and human toxicity (HT)

The biomass treatment stages, in which biomass undergoes saponification and solvent extraction, were the one that most influenced MET and HT. For these stages, great amounts of organic solvents were used (for the functional unit, 119 kg of hexane and 171 kg of ethanol for Scenario 1; 62.5 kg of methanol for Scenarios 1 and 2). While simple alcohols like methanol and ethanol, as well as alkanes such as heptane and hexane, are considered environmentally preferable solvents (Capello et al., 2007), their petrochemical production, use, and disposal involve toxicity-related impacts. The utilization of water-organic solvent mixtures (Amelio et al., 2014) or deep eutectic solvents (some of which can be derived from renewable resources) could be an attractive alternative, although industrial-scale data for this specific process are not yet available.

3.3. Depletion of fossil (FD), water (WD), and mineral (MD) resources.

The FD in our process was mainly due to the production of electrical energy. The electric energy consumed in these processes was supplied from the Spanish electricity grid, whose data in Ecoinvent for the Spanish mix corresponded to the period 2008-2015. During this period, the energy produced in Spain came mainly from non-renewable energy sources (coal, fuel/gas, combined cycle, etc.). The percentage of non-renewables during this period varied between 57.2% in 2014 and 78.4% (Red Eléctrica de España, 2015). Renewable energies, as well as nuclear, present CO_{2e} emission factors equal to zero. For their part, coal and fossil fuel power plants have an emission factor around 20 and 75 tCO_{2e} for MWh produced (REData, accessed 21st April 2023). This explains this great impact on our processes, although, looking at the geopolitical panorama that Europe faces in 2022, in addition to the upward trend in the increase in energy generation through renewable energies, it is expected that the energy mix of all countries, including Spain, be less dependent on fossil fuels in favor of cleaner energy sources.

Microalgal cultivation requires water (seawater or fresh water). Due to the shading of light by the biomass, concentrations of few grams per liter are expected (in industrial PBRs typically less than 1 g per liter). This means that to produce 1 kg of biomass, 1 m^3 of water is necessary only for the culture medium, although the water can be used in different cycles after being recovered in the centrifugation and/or sedimentation stages. In our case, when using a marine species, the water requirements for cultivation have less impact than those corresponding to freshwater species for obvious reasons. In addition, fresh water is commonly used as a cooling fluid, as well as for washing steps. Besides, water expense is implicit to all raw materials, as well as to electricity production (Mu et al., 2017). In the case of the processes studied here, it was one of the greatest impacts, since in addition to a significant amount of water used during the process, it was associated to other inputs. The WD can be reduced if wastewaters are used, not only for the water itself but also for the N and P contained in it, that would reduce the dependency on synthesized nitrate, phosphate, etc. (provided that the products obtained are not intended for human consumption). However, if the NH_3/NH_4^+ (or other components) concentration is too high, it will be necessary to carry out a dilution or even pretreatments (López Rosales et al., 2022). In addition, the use of wastewater makes it necessary to clean the equipment more frequently, which in turn is done with water (Mu et al., 2017). The use of seawater in processes such as cooling is not recommended since it is cause of major corrosion issues in pipes and pumping equipment. MD occurred mainly in the Inoculation & Culturing stage, due to the use of mineral fertilizers such as zinc sulfate, copper sulfate, or some phosphates, which create great impacts during the extraction of their raw materials (Ponomarenko et al. 2021; Valero, 2011).

3.4. Carbon footprint

For the evaluation of the carbon footprint, it has been considered that the CO_2 used for biomass production acted as a biogenic carbon source, thus treating the cultivation stage as a CO_2 sink. The carbon footprint values of each element used in Air.e LCA were collected as kg of CO_{2e} in Table 2. The results of the LCA showed that the production process of 1 L of fungicide has a carbon footprint of

104.87g CO_{2e}. If technical grade reagents instead of fertilizers had been used, the carbon footprint would have increased by only 0.24%. This impact (2.13 kg CO_{2e} in Scenario 1) was distributed among the different co-products being the impact of the fungicide the lowest. The carbon footprint obtained for the single-product fungicide in the Scenario 2 was 836.67 g CO_{2e}. This difference is associated with the allocation of burdens among the co-products based on their respective mass fractions, resulting in upstream stages contributing less to the functional unit. In Table 2 some carbon footprint values appear as negative figures. The reason is that impacts corresponding to the co-products, acted with respect to the fungicide as carbon sinks.

3.5. Comparison with commercial and non-commercial fungicides.

Regarding agriculture biofungicides, García-Cruz et al. (García Cruz et al., 2022) evaluated a fungicide obtained from the waxy residues of the production of orange essential oils. They made an LCA considering 1 m^3 of fungicide produced as functional unit. For comparative purposes we have normalized their results to our unit (1 L). In addition, LCAs have been carried out for different commercial fertilizers, based on the data provided by the maker in the safety data sheets (see supplementary materials). For all the fungicides in the comparison, 1 L of fungicide has been taken as the functional unit, and it has been recalculated, based on their recommended application concentration for one hectare of crop. In our case we have formulated our fungicide to dissolve 1 L of product in 200 L of broth. This is the amount of broth estimated to treat one hectare. This standardization is necessary since they have different concentrations and effectiveness. In this way, approximate estimates of their impacts evaluated under the different indicators are obtained. The values obtained are shown in Figure 5A. In the work of García-Cruz et al. (2022), the impact of ME was not calculated, so it is not represented in the Figure 5A. The case of the wax-based fungicide is also notable. A priori, a low impact would be expected due to its biological origin. However, it is not effective in low doses. Therefore, its use at high doses provides high values of GWP. GWP, HT and MD for the products with the lower impact were plotted in the Figure 5B. It was clearly showed that the fungicide produced in Scenario 1 was the one with the least environmental impact being at the level of Serifel, which is classified as a biological fungicide. Significant differences were obtained for the impacts ($p < 0.001$). Serifel is composed entirely of kaolin, a clay mineral that hardly requires post-processing in the manufacture of this product. The fungicide produced in Scenario 2 presented the greatest impacts in HT and MD. For traditional, chemical-based fungicides, the impacts related to toxicity, such as MET and HT, are much greater than for alternative fertilizers of biological origin, especially for the fungicide obtained in our Scenario 1. It is important to note that, to calculate these environmental data for commercial fungicides, their LCA has been carried out considering only their composition, obtained from their safety data sheets. Therefore, the actual impacts for this product are most probably greater since part of the manufacturing processes (formulation or packing) were not considered.

4. Conclusions

The proposed microalgal biofungicide under a biorefinery approach would imply the release of 34.61 t CO_{2e} during the useful life of the plant (15 years; total carbon footprint would be 703.91 t CO_2). The one-product scenario showed a larger carbon footprint (271.33 t CO_{2e}). When compared to commercial and non-commercial fungicides, the amphidinols-biobased fungicide showed a lower environmental footprint. The reduction in GWP was 82-98%. Especially significant was the reduction in the figures of the toxicity-related impacts, compared to commercial fungicides. Thus, from an environmental perspective, microalgal bioprocess are interesting alternatives with benefits that go beyond the reduction of the greenhouse gases.

E-supplementary data for this work can be found in e-version of this paper online.

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Figure Legends

Figure 1. Scheme of the methodological procedure and data sources used to produce 22,000 L/year of biofungicide from the culture of the marine dinoflagellate microalga *A. carterae.*

Figure 2. Overview of the process and system boundaries for Scenario 1 (A) and 2 (B). Background system groups raw materials and utilities whose environmental impacts were obtained from EcoInvent 3.7. Foregrounds system includes the environmental impact directly linked to the projected process.

Figure 3. Total impact of the scenarios evaluated for producing an amphidinol-based fungicide. Scenario 1 includes impacts of 3 co-products. Impacts of Scenario 2 are only referred to the biofungicide. Fossil Resource Depletion (FD), Mineral Resource Depletion (MD), Water Resource Depletion (WD), Global Warming Potential (GWP), Human Toxicity (HT), Marine Water Ecotoxicity (MET), Marine Eutrophication (ME) and Fresh Water Eutrophication (FE). Bars are standard deviations.

Figure 4. Comparison of contributions of the different process stages to the total impact for producing an amphidinol-based fungicide. Scenario 1 includes impacts of 3 co-products. Impacts of Scenario 2 are only referred to the biofungicide. Fossil Resource Depletion (FD), Mineral Resource Depletion (MD), Water Resource Depletion (WD), Global Warming Potential (GWP), Human Toxicity (HT), Marine Water Ecotoxicity (MET), Marine Eutrophication (ME) and Fresh Water Eutrophication (FE).

Figure 5. A) Environmental results of the fungicides evaluated normalized per treated ha. B) GWP, HT and MD for the 4 fungicides with lower impact. Scenario 1 and Scenario 2 are only referred to the biofungicide. Fossil Resource Depletion (FD): kg Oil_e, Mineral Resource Depletion (MD): kg $Cu_e \cdot 10^7$, Water Resource Depletion (WD): m^3, Global Warming Potential (GWP): kg CO_{2e}, Human Toxicity (HT): kg 1,4-DB_e, Marine Water Ecotoxicity (MET): kg 1,4-DB_e, Marine Eutrophication (ME): kg N_e and Fresh Water Eutrophication: kg P_e. Bars are standard deviations. *García-Cruz et al., 2022.

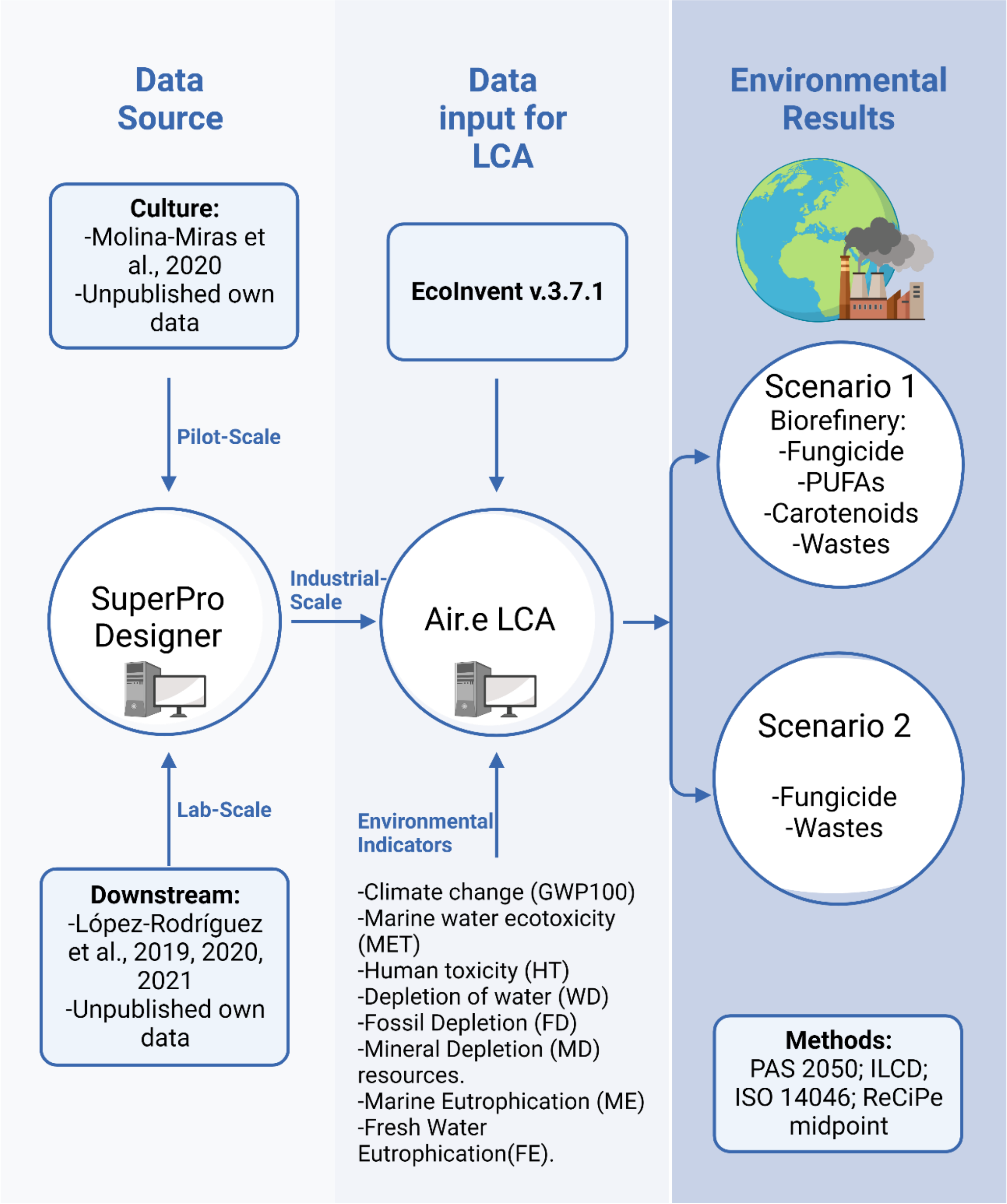
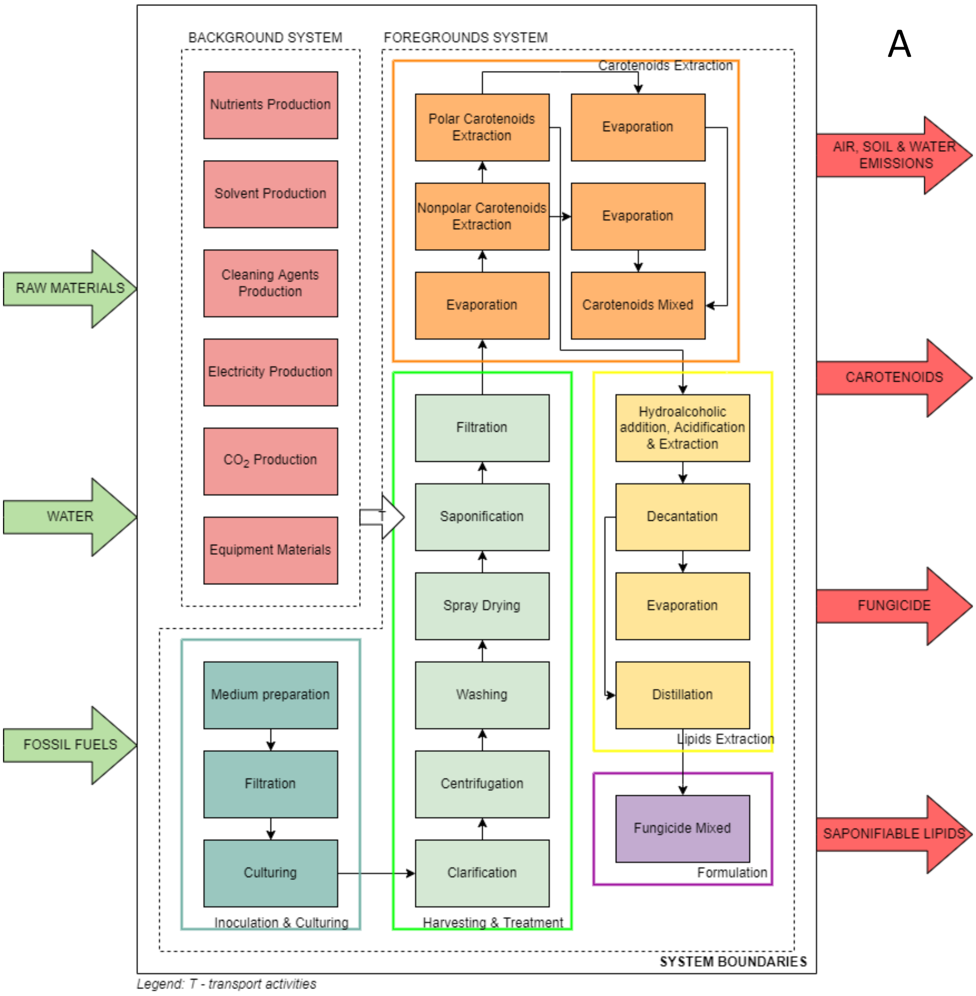

Data Source
Data input for LCA
Environmental Results
Culture:
-Molina-Miras et al., 2020
-Unpublished own data
EcoInvent v.3.7.1
Pilot-Scale
Scenario 1
Biorefinery:
-Fungicide
-PUFAs
-Carotenoids
-Wastes
SuperPro Designer
Industrial-Scale
Air.e LCA
Scenario 2
-Fungicide
-Wastes
Lab-Scale
Environmental Indicators
Downstream:
-López-Rodríguez et al., 2019, 2020, 2021
-Unpublished own data
-Climate change (GWP100)
-Marine water ecotoxicity (MET)
-Human toxicity (HT)
-Depletion of water (WD)
-Fossil Depletion (FD)
-Mineral Depletion (MD) resources.
-Marine Eutrophication (ME)
-Fresh Water Eutrophication(FE).
Methods:
PAS 2050; ILCD; ISO 14046; ReCiPe midpoint

Figure 1


A
BACKGROUND SYSTEM
FOREGROUNDS SYSTEM
Nutrients Production
Solvent Production
Cleaning Agents Production
Electricity Production
CO_2 Production
Equipment Materials
RAW MATERIALS
WATER
FOSSIL FUELS
Carotenoids Extraction
Polar Carotenoids Extraction
Evaporation
Nonpolar Carotenoids Extraction
Evaporation
Evaporation
Carotenoids Mixed
Filtration
Saponification
Spray Drying
Washing
Centrifugation
Clarification
Harvesting & Treatment
T
Hydroalcoholic addition, Acidification & Extraction
Decantation
Evaporation
Distillation
Lipids Extraction
Fungicide Mixed
Formulation
Medium preparation
Filtration
Culturing
Inoculation & Culturing
SYSTEM BOUNDARIES
AIR, SOIL & WATER EMISSIONS
CAROTENOIDS
FUNGICIDE
SAPONIFIABLE LIPIDS
Legend: T - transport activities

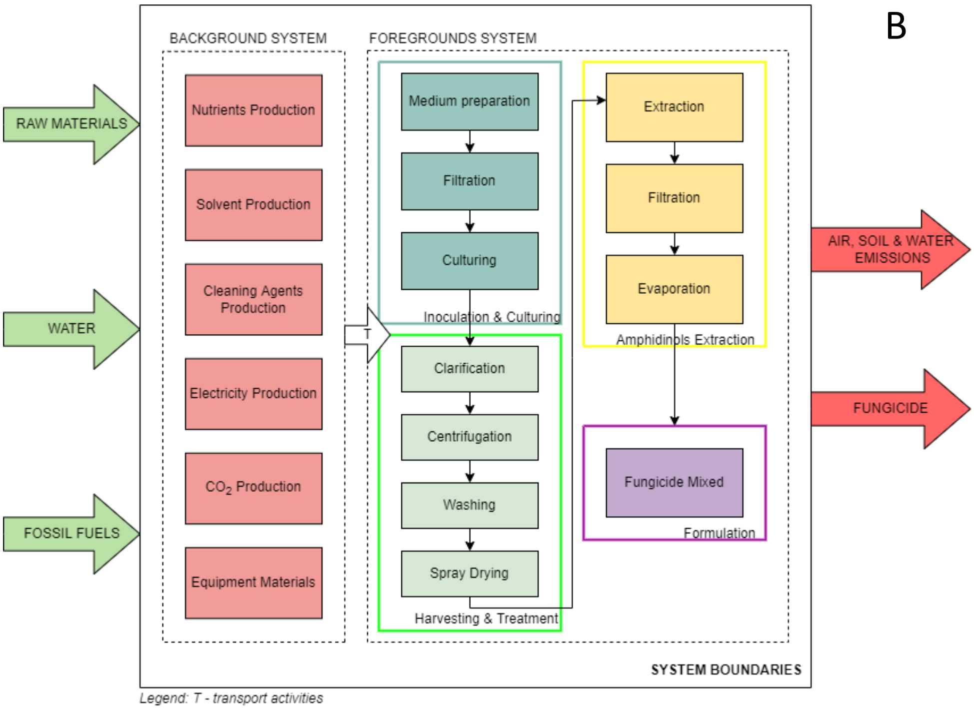
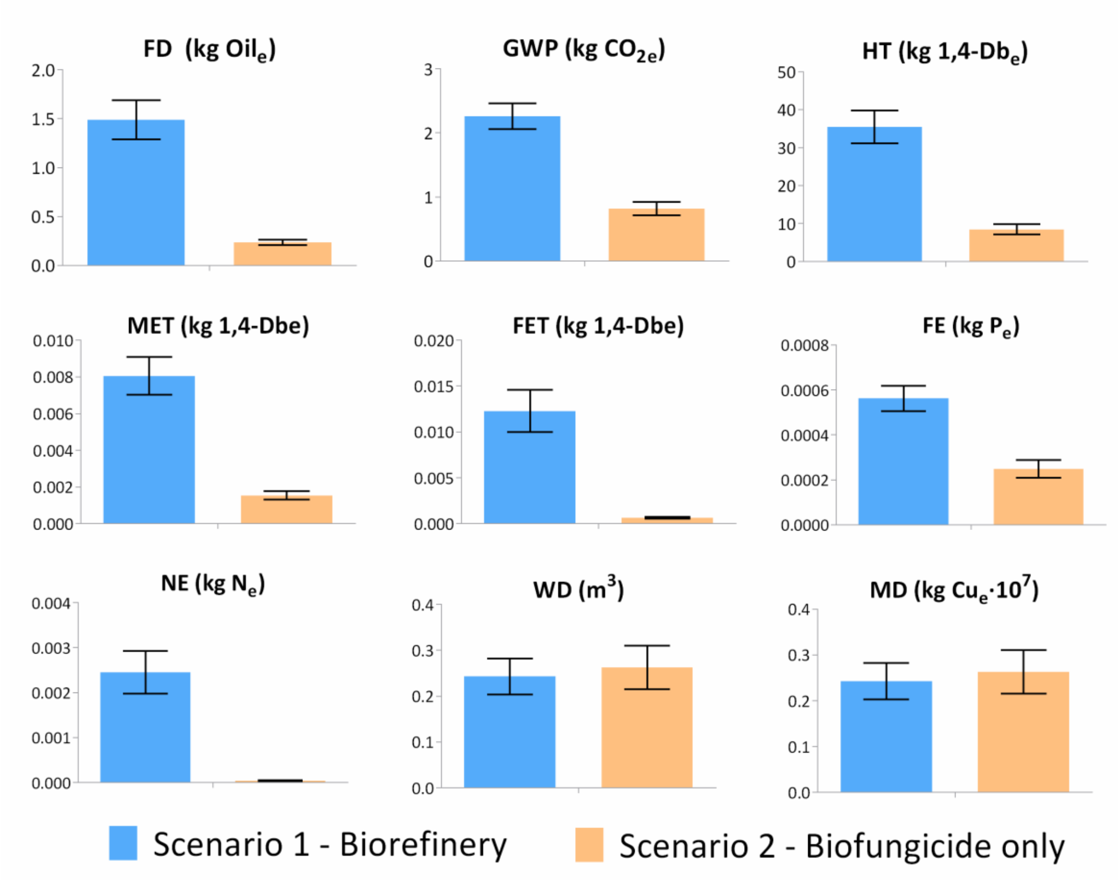

B
BACKGROUND SYSTEM
FOREGROUNDS SYSTEM
Nutrients Production
Solvent Production
Cleaning Agents Production
Electricity Production
CO_2 Production
Equipment Materials
RAW MATERIALS
WATER
FOSSIL FUELS
T
Medium preparation
Filtration
Culturing
Inoculation & Culturing
Clarification
Centrifugation
Washing
Spray Drying
Harvesting & Treatment
Extraction
Filtration
Evaporation
Amphidinols Extraction
Fungicide Mixed
Formulation
SYSTEM BOUNDARIES
AIR, SOIL & WATER EMISSIONS
FUNGICIDE
Legend: T - transport activities

Figure 2

681

682



683

684 Figure 3

685


FD (%)
FD (%)
GWP (%)
GWP (%)
HT (%)
HT (%)
MET (%)
MET (%)
FET (%)
FET (%)
FE (%)
FE (%)
NE (%)
NE (%)
WD (%)
WD (%)
MD (%)
MD (%)
Scenario 1
Scenario 2
Scenario 1
Scenario 2
Scenario 1
Scenario 2
1. Inoculation and Culturing
2. Harvesting
3. Carotenoids Extraction
4. Saponifiable Lipids Extraction
5. Fungicide formulation

Figure 4

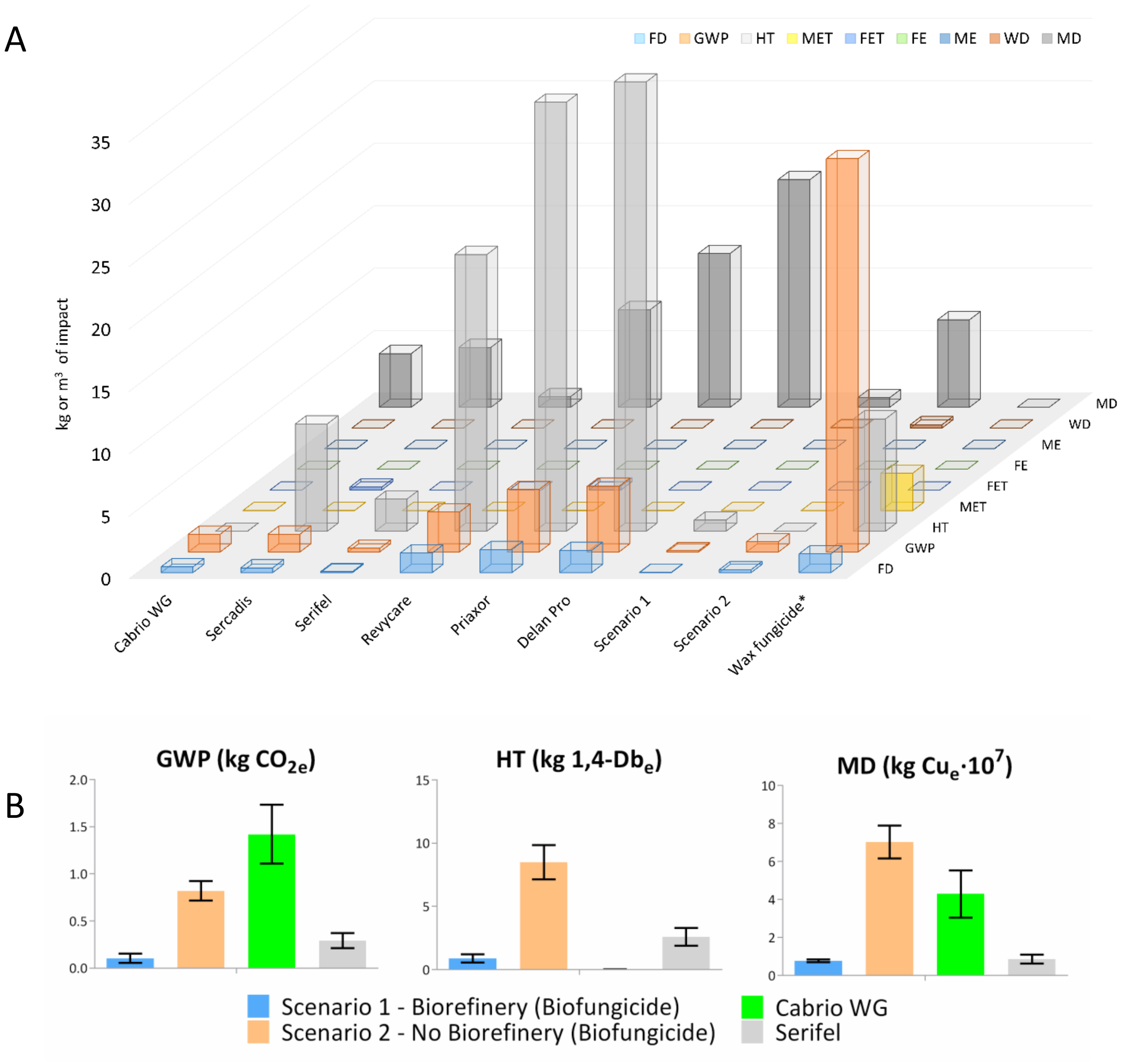

A
FD
GWP
HT
MET
FET
FE
ME
WD
MD
kg or m³ of impact
0
5
10
15
20
25
30
35
Cabrio WG
Sercadis
Serifel
Revycare
Priaxor
Delan Pro
Scenario 1
Scenario 2
Wax fungicide*
FD
GWP
HT
MET
FET
FE
ME
WD
MD
B
GWP (kg CO2e)
0.0
0.5
1.0
1.5
2.0
HT (kg 1,4-Dbe)
0
5
10
15
MD (kg Cue·10⁷)
0
2
4
6
8
10
Scenario 1 - Biorefinery (Biofungicide)
Scenario 2 - No Biorefinery (Biofungicide)
Cabrio WG
Serifel

Figure 5

Table 1. Inventory data for the amphidinol-based fungicide production process

Process	Inputs	Quantity	Outputs	Quantity
Common to both scenarios				
Inoculation & Culturing	Sea water (L)	8790	Purge (kg)	984.28
	Bleach (L)	30	Culture medium (L)	9611.03
	Thiosulfate (L)	7.5		
	Reagents (L)	107.78		
	Vitamins (g)	5.65		
	Inoculum biomass (g)	630		
	Sea water inoculum (L)	1000		
	Air (vvm)	0.5		
	CO_2 **(kg)**	643.66		
	Electricity (kWh)	95.09		
Scenario 1				
Harvesting & Treatment	Culture medium (L)	9611.03	Water (L)	9697.7
	Deionized Washing water (L)	104.41	Biomass (L)	14.03
	Potassium hydroxide (Kg)	1.58	Purge (kg)	0.24
	Ethanol (kg)	154.65	Solid waste (kg)	5.17
	Hexane (kg)	8.85	Mix to treat (kg)	229.62
	Electricity (kWh)	9.56		
Carotenoid Extraction	Inlet stream from previous step (kg)	229.62	Recovered tricomponent mixture (kg)	176.66
	Hexane (kg) (Nonpolar extraction)	52	Recovered hexane (kg)	49.4
	Acetone: Water (kg) (Polar extraction)	2.50	Heavy pase (kg)	4.6
	Water (g)	30	Recovered Acetone: Water (kg)	2.49
	Olive oil (L)	5.57	Stabilized carotenoids (L)	5.65
	Electricity (kWh)	14.302	Heavy phase-residue (L)	3.81
Lipid Extraction	HCl (L)	2.01		
	Hexane (L)	88.5	Recovered hexane (L)	60.05
	Ethanol: Water (L)	39.6	Recovered ethanol: water (L)	28
	Electricity (kWh)	2.51	Lipids (L)	0.71
Fungicide Formulation	Heavy pase-residue (L)	16.42	Fungicide (L)	92.78
	Water (L)	76.45		
Scenario 2				
Harvesting	Culture medium (L)	9611.03	Water (L)	9699.26
	Washing water (L)	100	Biomass (L)	12.47
	Electricity (kWh)	5.24		
Amphidinols Extraction	Methanol (kg)	62.53	Purge (kg)	0.13
	Biomass (L)	12.47	Solid waste (kg)	3.96
	Electricity (kWh)	5.725	Recovered methanol (kg)	60.89
			Broth rich in amphidinols (kg)	10.33
Fungicide Formulation	Broth rich in amphidinols (kg)	10.33	Fungicide (L)*	91.24
	Water (L)	80.65		

* The fungicide obtained in scenario 2 is more impure than that of scenario 1, given the presence of other compounds that have not been separated, such as saponifiable lipids and carotenoids.

Table 2. Carbon footprints (kg CO_{2e}) for an amphidinol-based fungicide process

Scenario 1		**Scenario 2**	
Elements	**kgCO_{2e}**	**Elements**	**kgCO_{2e}**
Inoculation & Culturing			
Reagent	$44.9\cdot10^{-3}$	Reagent	$45.13\cdot10^{-3}$
Vitamins	$22.1\cdot10^{-3}$	Vitamins	$22.1\cdot10^{-3}$
Electricity	$2.79\cdot10^{-1}$	Electricity	$6.42\cdot10^{-1}$
Compressed CO_2	$62.41\cdot10^{-3}$	Compressed CO_2	$64.21\cdot10^{-3}$
Harvesting & Treatment // Amphidinols extraction			
Water for washing	$369\cdot10^{-6}$	Water for washing	$378.73\cdot10^{-6}$
-	-	Methanol	$5.28\cdot10^{-3}$
Electricity	$9.28\cdot10^{-3}$	Electricity	$1.96\cdot10^{-2}$
Carotenoids Extraction			
Saponification	$338.72\cdot10^{-3}$		
Reagents	1.09		
Olive oil	$15.1\cdot10^{-3}$		
Cellulose	$170\cdot10^{-6}$		
Cardboard boxes	$2.42\cdot10^{-3}$		
Labels	$2.02\cdot10^{-3}$	-	-
Electricity	$12.15\cdot10^{-3}$	-	-
Saponifiable lipids Extraction			
Reagents	$369.27\cdot10^{-3}$	-	-
Electricity	$1.33\cdot10^{-3}$	-	-
Cellulose	$21.6\cdot10^{-6}$	-	-
Cardboard boxes	$902.64\cdot10^{-6}$	-	-
Labels	$7.5\cdot10^{-4}$	-	-
Fungicide formulation			
Water	$327\cdot10^{-6}$	Evaporation	$12.36\cdot10^{-3}$
Electricity	$4.22\cdot10^{-2}$	Water	$303.81\cdot10^{-6}$
Plastic bottles	$24.5\cdot10^{-3}$	Electricity	$0.92\cdot10^{-6}$
-	-	Plastic bottles	$24.55\cdot10^{-3}$
Labels	$3.71\cdot10^{-3}$	Labels	$3.73\cdot10^{-3}$
Cardboard boxes	$4.67\cdot10^{-3}$	Cardboard boxes	$5.04\cdot10^{-3}$
Carbon footprint/FU	***2.46***	***Carbon footprint/FU***	***0.85***