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Assessment of Tools and Methodologies for LCA and TEA of Advanced Biofuels: a preliminary case study on the SUN-PERFORM project

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S329808

A coloro che vivono senza ricordare,
e che amano solo con il cuore.
A mia nonna Anna,
la persona più buona del mondo,
che sarà la più contenta per me,
solo che poi non se lo ricorderà,
ma io sì.

Ti voglio bene

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ABSTRACT

The aviation sector, responsible for about 2.5% of global CO_2 emissions, is one of the hardest to decarbonize. A promising short-term solution is represented by Sustainable Aviation Fuels (SAFs). However, the integration of SAFs into the system still faces several limitations, including high costs and limited feedstock availability. Despite these challenges, several policies and organizations, such as ICAO and EU commissions, are promoting their adoption.

In this framework, the SUN-PERFOM project aims to solar fuel technologies, which currently face challenges such as low solar-to-fuel conversion efficiency (SFE ~0.50 %), low production rates and high investment and production costs. The project, through technological innovations and bio-engineering solutions, tries to overcome some of the existing limitations by producing lipids from microalgae. These lipids can be converted into SAF through ASTM-approved pathways (HEFA, CHJ, co-hydroprocessing of esters and fatty acids). Furthermore, the use of microalgae offers additional advantages, as they can be cultivated in both freshwater and saltwater, on non-arable land, and are capable of producing high lipid yields (up to 65-70% of dry biomass).

Since this technology has not yet reached commercial scale (TRL 4-7), a preliminary Life Cycle Assessment (LCA) plays a key role in identifying the project's hotspots and different scenarios will be analyzed to evaluate the potential of alternative solutions. To enhance competitiveness and sustainability, the project also includes co-product strategies based on waste valorization. Environmental impacts will be estimated, and in particular the potential reduction of greenhouse gas emissions compared to fossils.

For a more comprehensive study, a Techno-Economic Analysis (TEA) will be performed to estimate the costs of technology development. This will allow the recognition of the main cost drivers and economic constraints, providing inputs for strategic decision-making.

The final goal is to achieve a harmonized LCA and TEA that balance both environmental and economic dimensions. In addition, sensitivity analyses will support the identification of trade-offs between sustainability and feasibility.

As the project is in its early stages, this study provides a baseline to be refined with consortium data. At the current stage, and without accounting for technological innovations or engineered algae, from the preliminary LCA analysis, the GWP100 is estimated at ~396 gCO_2eq/MJ_{lipids} in Scenario A (exploiting a point source technology for CO_2 and a mix of wastewater and fertilizer for nutrients), while ~291 gCO_2eq/MJ_{lipids} in Scenario B (exploiting Direct Air Capture technology for CO_2 and fertilizers as nutrients). The analysis indicates that the largest contribution comes from fertilizers and energy consumption, in particular in the PBR, underscoring the need to reduce its energy demand and to find alternative to current fertilizers. Nevertheless, these values provide an initial benchmark,

and further reductions are expected with the implementation of the proposed innovations, thereby enhancing its commercial competitiveness.

Instead, from the TEA perspective, a preliminary production cost, based on a fully industrialized and high optimized facility, was estimated for the lipid fractions and resulted in ~1.5-3.4 €/kg_{lipids}. This preliminary value did not consider the possibility to valorize the residual part of the biomass, from which can be obtained high-value products and biochar, which can lead to economic benefits.

In future, a more comprehensive development of this analysis will be carried out within the framework of the SUN-PERFORM project, where a more complete study will be performed, also considering the residual part of the algae.

1 INTRODUCTION

This first chapter provides an overview of the current context of aviation field and the role of Sustainable Aviation Fuels (SAFs) in decarbonizing the sector. Starting with an analysis of the present state of aviation, highlighting its environmental impacts and the contribution of SAFs as an alternative to conventional jet fuels. A description of SAFs and their main production pathways is then presented, followed by a review of the key policies and regulations shaping their deployment at both international and national levels.

Building on this background, the SUN-PERFORM project is introduced as an innovative approach to producing biofuels from engineered microalgae. To assess the potential of this novel pathway, particular emphasis is placed on the role of Life Cycle Assessment (LCA) and Techno-Economic Assessment (TEA). These methodologies are essential for evaluating environmental impacts, economic feasibility, and overall benefits, providing the foundation for the analysis carried out in this work.

1.1 Current situation

Aviation sector has a significant impact on the environment and climate. It is a major contributor to greenhouse gas emissions, responsible for about 2.3–2.5% of global carbon dioxide emissions (in 2019) [1]. CO_2 emissions dropped sharply in 2020 due to the COVID-19 pandemic, falling by almost 50% compared to 2019 levels [Figure 1]. Since then, emissions have steadily recovered and, as of 2023, have reached around 90–92% of pre-pandemic levels, indicating a near-complete rebound of the aviation sector [2].

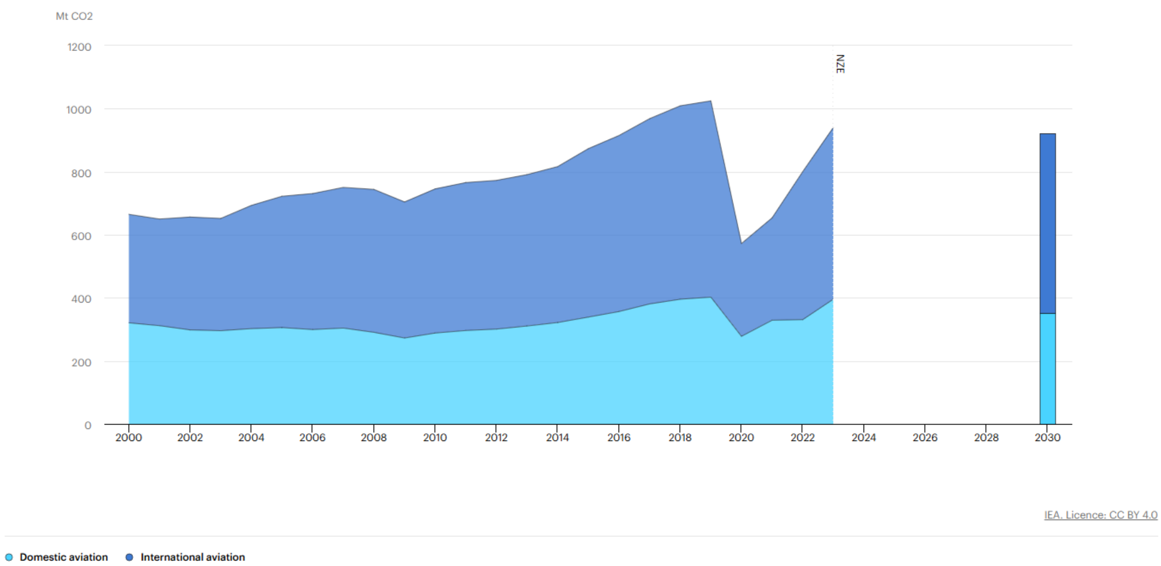


Figure 1 CO_2 emissions in aviation in the Net Zero Scenario, 2000-2030 [2]

Moreover, aviation also generates substantial emissions of nitrogen oxides and particulate matter, which intensify air pollution. With the continuous growth of the aviation market and the rising number of flights, these environmental impacts are becoming more severe, so it is essential to implement effective mitigation strategies and innovative solutions [3], [4], [5].

Sustainable aviation fuels (SAFs) represent a rapidly expanding strategy for decarbonizing aviation. The use of these fuels, due to their raw material sources and production processes, enables considerable decarbonization of aviation. SAF implementation can cut CO_2 emissions by 70–80%, particulate matter by 50–90% and nearly eliminate sulfur oxides. Moreover, they enable a reduction of nitrogen oxides through more efficient combustion. Additionally, their reliance on renewable resources further supports circular economy principles, where waste is transformed into valuable products [3], [4].

A wide range of feedstocks can be used for SAF production, such as plant biomass (vegetable oils, grains, sugar crops), organic residues and waste (used cooking oils, animal fats, forestry residues, straw), industrial and municipal waste (plastics, paper, coal by-products), recycled resources (waste gases), dedicated energy crops (camelina, miscanthus), and microorganisms like microalgae. Consequently, fuel generation becomes independent of petroleum, whose reserves are limited [3].

A notable advantage of SAF implementation is that it does not require modifications to airport infrastructure or aircraft fleets, enabling airlines to adopt these fuels rapidly and with limited investment. This transition supports compliance with sustainability standards set by aviation bodies, contributes to climate and environmental goals, and addresses the growing expectations of society, regulators, and investors for reduced carbon footprints. Moreover, SAF use facilitates the fulfillment of international commitments, improves access to funding, strengthens corporate reputation, and fosters customer trust. In this way, SAF enables the aviation sector to pursue economic growth while advancing sustainable development and environmental protection [4].

Despite their potential, the large-scale adoption of sustainable aviation fuels faces several challenges and limitations. One of the main obstacles is their high production cost, which remains significantly above that of conventional jet fuels. The advanced technologies involved, together with complex processes and infrastructure, make both investment and maintenance expensive. Moreover, to achieve the desired emission reductions, SAF production must be scaled to an industrial level and adopted globally, rather than being limited to specific regions or companies. Another constraint is the feedstock availability, as many of the raw materials also serve the food, energy, agricultural, and road biofuel sectors, creating competition that lead to increase market value and acquisition costs [4], [5], [6].

Airlines are often reluctant to adopt SAF because of its limited economic profitability. In response, international aviation bodies and governments have introduced several initiatives to promote its development. These measures are principally financial, such as grants, investment schemes, and tax incentives. But they also support research and development aimed at scaling production, improving efficiency, and broadening the range of feedstocks while lowering costs. In parallel, regulations and standards are being introduced to guarantee full compatibility with existing infrastructure and aircraft fleets. Several programs have been developed to promote SAF as a solution to reduce dangerous emissions, while others impose minimum SAF usage requirements for airlines [4].

Sustainable Aviation Fuels (SAF) are currently considered a key element in the decarbonization of the aviation sector, proposing a feasible solution to reduce life cycle greenhouse gas emissions without requiring significant modifications to aircraft or infrastructure. While SAFs can significantly reduce aviation-related emissions with respect to fossil jet fuels, the magnitude of benefits depends mainly on supply chain design and sustainability criteria. There are different certified pathways for SAF production, along with recent technologies that have the potential to contribute more to the development of the industry. Otherwise, the adoption of SAFs on large scale is limited by costs, infrastructures, and regulations. Thus, although SAFs represent a promising pathway for aviation climate mitigation, substantial scaling efforts, regulatory support, and continued technological innovation are essential to achieve their full potential [7].

1.2 Sustainable Aviation Fuels

Sustainable Aviation Fuels (SAFs) are a safe replacement for conventional fuels (fossil-based) that can reduce greenhouse gas emissions. SAFs are almost chemically identical to traditional jest fuel but are produced from feedstock that absorbs CO_2 and so allow a net reduction in emissions if compared to fossil fuels. SAF can be produced from different feedstock and various processes that will be analyze later. Other terms that have a meaning similar to SAF are: biofuel, renewable aviation fuels, renewable jet fuel, and bio jet fuel [8].

SAF description includes three key elements [8]:

- Sustainability refers to low-carbon feedstock that can be sourced continuously in a way that aligns with economic, social, and environmental objectives. It requires maintaining ecological balance by preventing the depletion of natural resources, avoiding competition with essential needs such as food, land, water, and mitigates the aviation sector's impact on climate change;
- It is an alternative to traditional energy sources for aviation, in this case non-conventional or advanced fuels. Unlike fossil-based fuels such as oil, coal, or natural gas, these fuels are produced through alternative processes to generate jet fuels. SAF feedstocks are various and

include used cooking oil, plant oil, municipal waste, waste gases, agricultural residues, green hydrogen, and even electricity. However, it is important to highlight that not all alternative fuels qualify as "sustainable";

- Aviation fuel refers to drop-in fuels that comply with the technical standards for use in commercial aircraft and is compatible with existing engines and fuel systems, ensuring that safety, one of the most important aspects, is maintained.

The International Civil Aviation Organization (ICAO) defines SAF as renewable or waste-derived aviation fuels that meets sustainability criteria reported in ICAO Carbon Offsetting and Reduction Scheme for International Aviation (CORSIA) [9].

Instead, according to article 3 of the ReFuelEU Aviation regulation [10], SAF is defined as a drop-in fuel that meets the sustainability requirements established in the Renewable Energy Directive (RED) [11]. SAF are defined as [12]:

- Synthetic aviation fuels mean aviation fuels that are 'renewable fuels of non-biological origin' (RFNBOs) and limited to drop-in fuels only;
- Advanced biofuels produced form feedstock listed in Part A of Annex IX in the meaning of Article 2(34) of RED;
- Biofuels derived from feedstock listed in Part B of Annex IX, in the meaning of Article 2(33) of RED;
- Recycled Carbon aviation fuels in the meaning of Article 2(33) of RED.

1.2.1 Certified pathways

To be considered as a real alternative to fossil jet fuel, SAF has to respect specific quality characteristics. Two technical norms regulating the sector have been released by the American Society for testing and Materials (ASTM): ASTM D4054 (Standard Practice for Qualification and Approval of New Aviation Turbine Fuels and Fuel Additives) outlines the process required for an alternative fuel to be approved for use under ASTM D7566-17a (Standard Specification for Aviation Turbine Fuel Containing Synthesized Hydrocarbons). The procedure does not address the quality of the fuel, but the path required for its production [3].

Currently eleven production pathways have been fully certified for blending with fossil aviation jet. These aviation biofuels are drop-in which means that they can be directly blended with fossil (ASTM D1655: Specification for Aviation Turbine Fuels) but with differing blend limits [3], [13].

In **Table *1*** are summarized all the pathways already certified [13].

Table 1 Conversion processes already certified by ASTM

ASTM reference	Conversion process	Abbreviation	Possible Feedstocks	Max Blend Ratio
ASTM D7566 Annex A1	Fischer-Tropsch hydroprocessed synthesized paraffinic kerosene	FT	Coal, natural gas, biomass	50%
ASTM D7566 Annex A2	Synthesized paraffinic kerosene from hydroprocessed esters and fatty acids	HEFA	Vegetable oils, animal fats, used cooking oils	50%
ASTM D7566 Annex A3	Synthesized iso-paraffins from hydroprocessed fermented sugars	SIP	Biomass used for sugar production	10%
ASTM D7566 Annex A4	Synthesized kerosene with aromatics derived by alkylation of light aromatics from non-petroleum sources	FT-SKA	Coal, natural gas, biomass	50%
ASTM D7566 Annex A5	Alcohol to jet synthetic paraffinic kerosene	ATJ-SPK	Ethanol, isobutanol and isobutene from biomass	50%
ASTM D7566 Annex A6	Catalytic hydrothermolysis jet fuel	CHJ	Vegetable oils, animal fats, used cooking oils	50%
ASTM D7566 Annex A7	Synthesized paraffinic kerosene from hydrocarbon - hydroprocessed esters and fatty acids	HC-HEFA-SPK	Algae	10%
ASTM D7566 Annex A8	Synthetic Paraffinic Kerosene with Aromatics	ATJ-SKA	C2-C5 alcohols from biomass	
ASTM D1655 Annex A1	co-hydroprocessing of esters and fatty acids in a conventional petroleum refinery		Vegetable oils, animal fats, used cooking oils from biomass processed with petroleum	5%

ASTM D1655 Annex A1	co-hydroprocessing of Fischer-Tropsch hydrocarbons in a conventional petroleum refinery		Fischer-Tropsch hydrocarbons co-processed with petroleum	5%
ASTM D1655 Annex A1	Co-Processing of HEFA	Hydroprocessed esters/fatty acids from biomass'		10%

Moreover, SAF must undergo strict laboratory, ground, and flight test under an internationally-recognized standard, this to validate its performance and guarantee full compliance with aviation requirements [8].

Now this section examines in greater detail the pathways for the production of SAF.

- *Fisher-Tropsh Hydroprocessed synthetized paraffinic kerosene (FT-SPK):* is produced through gasification, a thermochemical process that converts biomass or other solid feedstocks—such as residual or organic waste—into gas. The process then proceeds with syngas purification and conditioning, during which contaminants like sulfur compounds, nitrogen oxides, hydrogen chloride, and particulates are eliminated to avoid catalyst deactivation in subsequent fuel synthesis. The purified syngas is then enriched with hydrogen to achieve the optimal hydrogen-to-carbon monoxide ratio. The core of the process is the Fischer–Tropsch synthesis, where hydrogen and carbon monoxide react over a catalyst to form long-chain hydrocarbons that serve as the foundation of synthetic fuels. The target output typically includes C_{10}–C_{20} hydrocarbons, given their suitability for further refining. These hydrocarbons undergo hydrorefining to improve their chemical properties, followed by distillation to separate fractions based on boiling points. Finally, the resulting fractions are blended in defined proportions to produce synthetic paraffinic kerosene (SPK), which is subsequently mixed with conventional Jet-A1 fuel [7]. It guarantees a high flexibility of feedstock, it is Sulphur free and presents low aromatic. On the other hand, it requires high consumption of electricity, presents rigorous condition for syngas production and a dispersed production [4].
- *Synthesized paraffinic kerosene from hydroprocessed esters and fatty acids (HEFA):* Some advantages are that it is possible to use it without blending with petroleum-based jet fuel and presents the highest energy conversion efficiency among all SAF production routes. However, it is restricted to oil-derived feedstock [4].

- *Synthesized iso-paraffins from hydroprocessed fermented sugars (SIP):* Relies on the conversion of sugars derived from the processing of genetically modified microorganisms such as algae, fungi, yeast, and bacteria. It is characterized by a low emission of pollutants and a similar combustion behavior with Jet A1 fuel. But presents an high viscosity and a poor combustion, requires expensive feedstock and high energy consumption, has the lowest blending ratio limit [4].
- *Synthesized kerosene with aromatics derived by alkylation of light aromatics from non-petroleum sources (FT-SKA):* FT-SKA represents a modification of the FT-SPK pathway. Although the primary production steps—syngas generation, Fischer–Tropsch synthesis, and hydrorefining—are identical, the end product is distinguished by the incorporation of aromatic hydrocarbons. The key difference is the blending Fischer–Tropsch–derived synthetic kerosene with aromatic compounds (such as benzene, toluene, and xylenes) obtained from biomass [7]. It is able to achieve up to 20 wt% of alkylated aromatics and can be used without blending with petroleum-based jet fuel, but it is restricted to coal tar feedstock [4].
- *Alcohol to jet synthetic paraffinic kerosene (ATJ-SPK):* involves converting alcohol into synthetic paraffinic kerosene. It provides method to produce aromatic hydrocarbons, although is restricted to ethanol and isobutanol feedstock, high-value alcohol are required as feedstock and presents a lower energy density [4].
- *Catalytic hydrothermolysis jet fuel (CHJ):* The feedstocks for this method consist of vegetable oils, animal fats, and used cooking oils, similar to those utilized in the HEFA pathway. The procedure starts with a preconditioning phase designed to modify the structure of fatty acid molecules and thereby enhance the efficiency of the subsequent thermolysis process [7]. Has an identical molecular composition and chemical constituents of petroleum crude but require an high energy consumption [4].
- *Synthesized paraffinic kerosene from hydrocarbon - hydroprocessed esters and fatty acids (HC-HEFA-SPK):* utilizes algae, which has a higher growth rate compared to other agricultural crops, nevertheless presents a low blending limit [4]. With this process hydrocarbon components are introduced through sources like algae. Then these components are processed into esters and fatty acids, which are further hydrorefined. This combined approach increases fuel properties and production efficiency [7].
- *Synthetic Paraffinic Kerosene with Aromatics (ATJ-SKA):* is a variant of the ATJ process, distinguished by the addition of aromatic compounds—benzene, toluene, and xylenes—after base hydrocarbon formation. These aromatics enhance energy density, lubricity, and chemical stability of the fuel [7].

- *Co-Hydroprocessing of esters and fatty acids in a conventional petroleum refinery:* it co-processes biological feedstocks (plant oils and animal fats) with petroleum in already existing refinery infrastructure. After purification there is a step in which esters and fatty acid are hydrorefined and isomerized and then the product is distilled and blended with conventional jet fuel [4].
- *Co-Hydroprocessing of Fischer-Tropsch hydrocarbons in a conventional petroleum refinery:* In contrast to standalone FT-SPK production, this approach incorporates Fischer–Tropsch products into existing refinery streams, allowing simultaneous processing with crude oil. This pathway leverages existing infrastructure, reduces capital costs, and limits harmful emissions [3], [4]. Moreover, it produces lower-carbon intensity fuels, allows to use existing infrastructure from petroleum refineries, is able to handle different biogenic feedstock. Although, requires oxygen removal process and there are difficulties in operational due to factors such as type of biogenic feedstock, blend rates and product characteristics [4].
- *Co-Processing of HEFA (Hydroprocessed esters/fatty acids from biomass):* resembles the conventional HEFA process but differs in that biological feedstock are processed together with crude oil in existing refineries. Although HEFA, hydroprocessing, and HEFA co-processing are interrelated, their configurations are different. HEFA co-processing employs pyrolysis or thermal liquefaction to generate bio-oils from biomass feedstocks, which are subsequently subjected to the HEFA pathway prior to blending with conventional petroleum-based jet fuel [4].

1.2.2 Feedstock

The choice of feedstock is essential in determining the distinct category of aviation fuel, as it depends on both its origin and characteristics, especially in the context of SAF. To be considered suitable for SAF production, feedstock must meet certain requirements. The feedstock should enable a high SAF yield and should: (I) be available (II) does not contend with food production, (III) be cost-effective, and (IV)presents low environmental footprint.

The first requirement is the feedstock availability. Up to now, around 95% of global biodiesel is produced from edible oils, which are readily sourced from large-scale agriculture. Nevertheless, this practice utilizes food resources toward fuel, raising concerns about food security and market supply. Therefore, SAF feedstocks should avoid competing with food production. They must also be cost-effective and exhibit a low carbon footprint, with limited land use. Furthermore, relying on food crops can result in land use changes, such as deforestation and ecosystem destruction, and can also lead to an increase in food prices due to resource competition. Additionally, indirect impacts from irrigation, pesticides, and fertilizers may further harm the environment and increase greenhouse gas emissions [4].

In the Figure 2 are summarized the different feedstock for SAF production. Here a brief description [4].

- *First generations* are produced from edible crops such as palm oil and corn. Although these feedstocks are easily available, their use raises concerns due to the competition with food resources, limiting their potential for large-scale biofuel production. For this reason, first-generation fuels, being derived from food crops, are excluded from SAF options as they are not considered sustainable;
- *Second generation* originate from non-edible sources that do not compete with food production. However, as these materials primarily consist of residues and wastes, their utilization is often constrained by variability in quality and by an inconsistent supply;
- *Third generation* are typically non-food sources with higher potential for efficiency and sustainability. They include plants and algae cultivated specifically for aviation applications, making them suitable candidates for SAF production.

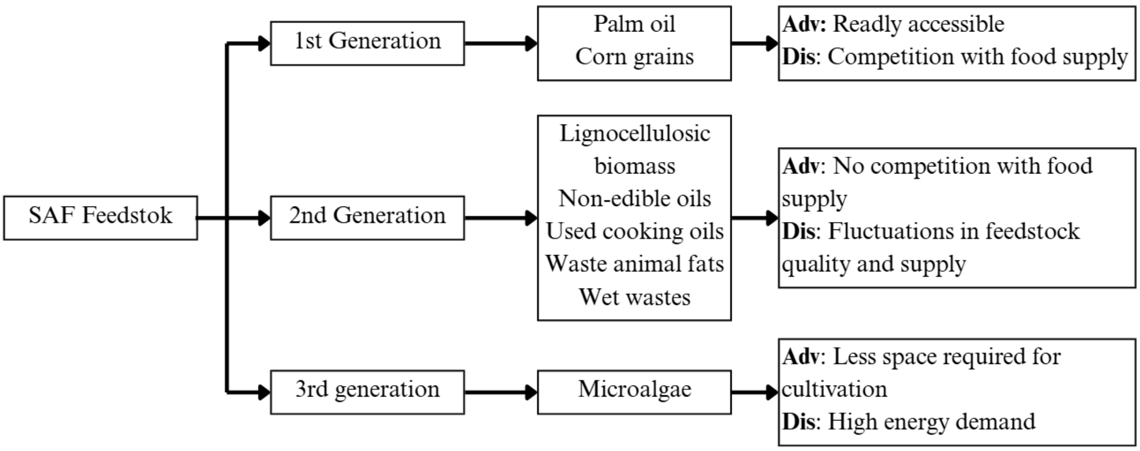


Figure 2 Scheme of possible feedstock for SAF production

1.3 Policy and regulations

SAF policies differ across countries due to variations in feedstock availability, infrastructure, production technologies, and regulatory priorities, as well as local economic and social conditions. SAF are promoted more effectively through public pressure and consumer expectations. Moreover, collaboration among countries, organizations, and businesses is necessary to achieve common goals in stopping climate change through greenhouse gas reduction and supporting sustainable development [4], [7].

To guarantee the development and distribution of SAF, several restrictions have to be addressed. If the production cost of SAF, including the advanced production infrastructure and feedstock availability, is higher than the fossil kerosene then SAF production and consumption will not be promoted. Thus, policy must be carried out to advance SAF production. Through the implementation of policy options, SAF's economic barriers can be addressed. There are three pillars that denote the effectiveness of a policy, (I) Practicability, in which the focus lies on achieving concrete policy outcomes rather than remaining at the level of ideas or theories, (II) Feasibility where the policy can be easily implemented and (III) effectiveness where the desired results are successfully achieved. Indeed, establishing enduring and reliable policies is crucial to establishing a stable market for SAF [4].

ICAO

The International Civil Aviation Organization (ICAO) is in charge coordinating and regulating international civil aviation. It defines standards and recommendations for all aspects of air transport, with particular emphasis on environmental protection and sustainable growth. ICAO actively promotes the development of alternative fuel concepts and plays a central role in their promotion. In cooperation with governments, research bodies, and industry, it works to implement SAFs on large scale. The organization's policy is structured around three main phases.

The first stage focuses on promoting Sustainable Aviation Fuels (SAF). ICAO and governments support the sector through funding, tax incentives, and measures to reduce investment risks. Resources are allocated to R&D in universities, research centers, and private companies, as well as to infrastructure for SAF production and distribution. Incentives include tax credits, loan guarantees, exemptions from corporate taxes, and accelerated depreciation, along with bonds issued by public and supranational bodies. Additional reductions apply to SAF excise duties, producers, suppliers, and blending entities. These measures aim to equalize cost differences between fossil fuels and sustainable alternatives. Farmers and feedstock providers also receive support to establish new crops. Additionally, SAF use may generate further benefits through exemptions or reductions in carbon taxes and emission-based charges.

Secondly, it focuses on stimulating demand for SAF by integrating it into current aviation industry. In this stage, governments play a central role by introducing mandates that require the use of SAF and reduce dependence on traditional fossil-based jet fuels. Furthermore, government should demonstrate leadership by setting ambitious carbon reduction targets through SAF adoption and in promoting its application within governmental and military transport systems.

The third phase sees the government taking on a facilitating role by certifying SAF production, assisting in the creation or validation of relevant systems, and supplying policymakers with critical information and feedback. Moreover, Coordination of efforts and sharing information will be guaranteed through collaboration among stakeholders involved and discussion with industry representatives. [4]

During the 39th session of ICAO assembly (2016), an agreement on a Global Market Based Measure (GMBM) to address international aviation emissions have been found. The aim is to offset emissions exceeding 2020 levels by investing in green projects and programs. The Carbon Offsetting and Reduction Scheme for International Aviation (CORSIA) started with a pilot and voluntary phase in 2021 for all ICAO member states, and is expected to become mandatory from 2027, although emissions data should be collected from 2019. [3]

In conclusion, ICAO supports in an active way the implementation of SAF, focusing on three main areas. Through the collaboration with governments, research institutions, and the private sector, it defines a global framework that accelerates the transformation of the aviation sector towards sustainability [7].

The European Union

The European Union actively supports environmental protection efforts in aviation sector. It developed a range of regulations and policies as part of its decarbonization plan. EU also creates programs which aim is the reduction of harmful emissions. One of the proposed solutions is the implementation of renewable SAFs.

One of the EU's initiatives introduced is the "Fit for 55" climate policy package, whose principal goal is to cut greenhouse gas emissions by 55% by 2030 respect to 1990 levels. This is included in the European Green Deal strategy, which aims to achieve climate neutrality by 2050. The "Fit for 55" package includes the ReFuelEU Aviation initiative regarding SAF, as the single most powerful tool to decrease aviation CO2 emissions. By means of its implementation, the goal is to gradually increase the share of SAF blended into the conventional aviation fuel supplied at EU airports. The planned targets are to reach 2% usage of SAF from 2025 and increase this to 70% share of SAF from 2050. It also targets the minimum share of synthetic aviation, 1.2% from 2030 and increase this to 35% from 2050. The successful implementation of ReFuelEU Aviation requires the collaboration of three principal sectors: aviation fuel suppliers, EU airports and airlines [10], [12].

ReFuelEU Aviation introduces a single set of EU rules with harmonized targets for the supply of SAF, allowing fuel suppliers, airports, and airlines to operate under equal conditions across the EU. By replacing national mandates, the initiative provides clear signal and market certainty, while reducing compliance costs for all actors. It also encourages the creation of global value chains, since SAF, its feedstock, and the production technologies can be developed worldwide with positive socio-economic impacts. Greater production and uptake of SAF will, over time, lower prices thanks to increased competition, economies of scale, and improved production efficiency. ReFuelEU Aviation is expected to stimulate innovation and investment throughout the whole supply chain and in all EU regions, as SAF can be produced in every EU Member State. Finally, the initiative will support job creation and cohesion across Europe, since new SAF production facilities will be established throughout the Union and SAF will be made available at every EU airport [10].

Another measure adopted by the European union to limit greenhouse gas emissions is the development of the Emissions Trading System (ETS). For the sectors with the highest CO_2 emissions, this program sets a cap on total emissions, requiring companies to receive or purchase allowances accordingly. Companies emitting more than their quota must purchase extra allowances from other companies, while those below the limit can sell their surplus. So, companies that remain under their emission limits benefit financially from their environmentally friendly approach, while those that damage the environment sustain additional costs. Hence, this should encourage companies to adopt more eco-friendly solutions. Over time, allowances gradually decrease, businesses are pushed to invest in sustainable technologies. In this framework, the use of SAF is particularly advantageous, as it reduces emissions and helps avoid additional costs [5], [7], [14].

Moreover, the European Union actively promotes research and development. It supports both internal and local initiatives focused on climate protection. It runs the Horizon Europe research and innovation program, in which substantial funding is provided for scientific research, aimed at identifying new raw materials, enhancing production processes, and developing the infrastructure required. Grants are achievable also for the construction or modernization of infrastructure elements required for fuel production, refueling, blending, or other activities related to the SAF production process. In this way, investors are encouraged to implement new technologies more quickly since the financial risk is reduced [7], [15].

Great importance is given to building alliances and partnerships between countries as well as between public and private actors, since such cooperation leads to multiple benefits. The European Union, for instance, works with ICAO to standardize SAF certification rules and regulations. The goal is to ensure that fuels produced worldwide comply with uniform standards.

Summing up, the European Union undertakes numerous actions to protect environment in aviation, introducing various rules, regulations and incentive programs related to sustainable fuels. The main goal is decarbonization and achieving climate neutrality [7].

IATA

The International Air Transport Association (IATA) promotes the development of sustainable aviation fuels, considering them as the most effective way to achieve a significant reduction in emissions. In 2009, it set the target of halving aviation-related emissions by 2050 compared to 2005 levels. IATA also urges governments to offer financial support to companies and encourages cooperation and partnerships among stakeholders [7].

Other Entities – Governments, Enterprise, Airlines

Since the urgency to reduce greenhouse gas emission is high, not only major environmental organizations but also governments and private companies are gaining awareness of the need for a change and so are joining forces to protect the environment.

SAF policies adopted by other entities regarding SAFs are analyzed in "Sustainable Aviation Fuels: A Comprehensive Review of Production Pathways, Environmental Impacts, Lifecycle Assessment, and Certification Frameworks [7] ", here **Table *2*** is reported an overview:

Table 2 SAF policies adopted in different countries

Country/Region	Policy/Iniziative	Targets/Investments
Portugal	Roadmap for Carbon Neutrality 2050	Eliminate aviation CO_2 by 2050
Spain	Climate Law 2020	2% SAF by 2025
France	Climate Law 2020	5% SAF by 2030
Sweden	Fossil-Free Industry Initiative (2020)	30% SAF blending
Malaysia	-SAF from palm oil funding -Blending mandates (2007 → 20% by 2024)	- USD 6.8M investment - Raise blending 5% → 20% (2024)
Brazil	-National Biofuels Policy (RenovaBio) -Research: HTL with hydrogenation for SAF (85% GHG cut)	- Promotes decarbonization and carbon credits market - Boost SAF with sugarcane + HTL
UK	Renewable Transport Fuel Obligation (2018)	Support SAF producers
Netherlands	First SAF plant (2019)	First production plant
Indonesia	-National Action Plan (blending targets -Archipelagic Carbon Program - Expanded HEFA palm oil biofuel (2022)	- 2% SAF (2016), 3% (2020), 5% (2025) - Carbon credit scheme - 3,000 M barrels/day
Japan	Next-Generation Aviation Fuel Initiative (2014)	Develop SAF production and showcase SAF use at Tokyo 2020 Olympics
USA	-Renewable Fuel Standard 2 - WasteFuels project (company)	-Obligation for fuel producers/importers

	- U.S. Department of Agriculture, Boeing, and airlines	-SAF form municipal and agricultural waste -1 billion gallons of biofuels annually
California (USA)	-Low Carbon Fuel Standard -Sustainable Aviation Fuel Act	-Credit trading for SAF -Tax incentives for SAF
Airlines	-SAF blending (2022) (Indian airline) -SAF development (other airlines)	-1% blending start -research, investment, pilot projects and test flights
France (Industry)	Global Bioenergies -TotalEnergies	-SAF plant 30,000 t/y (2027), -SAF plant 150,000 t/y (2030, 10% global share)
Thailand	SAF-dedicated plant Bangchak Phra Khanong Refinery (2024)	1M barrels/day production
Philippines	Domestic bioethanol from sugarcane	Bioethanol (sugarcane), SAF from waste
Colombia	Biofuels: ethanol (sugarcane), biodiesel (palm oil, residues, waste)	Certified feedstocks (RSB/ISCC), pathways HEFA, FT, ATJ
Qatar	Pilot projects scaling up to 1.5M liters/year	Scaled pilot projects
ICAO	CORSIA offset scheme	Offset 80% of emission growth (2021-2035)

1.4 SUN-PERFORM project

As already mentioned in the “Policy and regulations” section, to achieve global climate goals and the European Green Deal targets, affordable CO_2-neutral fuel is crucial. The European Commission plans to decarbonize aviation by raising sustainable fuel use at airports from 2% in 2025 to 70% by 2050 [10]. Current alternative fuels face challenges such as high costs, feedstock limited availability, land use competition, low energy-conversion rates and poor performances in their production chain.

In alternative fuels, solar fuels are the most recent that emerge. They have enormous potential, but low solar-to-fuel conversion efficiency, low production rates and prohibitively high costs are hindering their potential. Thus, to achieve sustainable and affordable solar fuels, breakthroughs in technological innovation are essential for advancing the field.

Sun is the ultimate and widely spread energy source for our planet. However, natural photosynthetic efficiency (PE – sunlight conversion to biomass), which is the direct process to convert solar energy into chemical energy, is a key limiting factor to realize a feasible solar fuel production. Among the factors that contribute to this inefficiency there are: the limited capture of the solar light spectrum by biological photosynthesis, slow enzymatic conversions and high energy consumption during the CO_2 fixation and downstream cellular metabolism.

Between the different types of feedstocks, microalgae are relatively fast-growing photosynthetic microorganisms that can be grown in both fresh or sea water, in open or contained photobioreactor, placed on non-arable land (hence avoiding deforestation). Their PE reaches 2,5% under industrial cultivation conditions, and so, compared to others agricultural crops that usually reaches only ~1% or below, are the most efficient in the conversion of solar light into biomass [16].

Microalgae can directly produce lipids at high quantities, above 50% of their mass. Moreover, Microalgal TAG lipid-based fuels are already approved as direct production routes for Sustainable Aviation Fuel (SAF) [13]. Hence, microalgae stand out as highly promising platform for aviation solar fuel.

The SUN-PERFORM project (**S**ynthetic biology **U**nited with **N**anotechnology – A Biohybrid Approach to Improve Light harvesting and CO_2 fixation for high **Perform**ance Sustainable Solar Fuel Production) [17] in response to these challenges, integrates multiple scientific disciplines and experts towards the common goal to unlock new frontiers in solar fuel production. The project aims to create a biohybrid route from solar energy to fuel. This innovative pathway involves the integration of an artificial light- harvesting system with advanced microalgal solar cell factories. In addition to the technical advancement, a complete evaluation on the sustainability, techno-economic and social aspect will be done on this novel route.

The project objectives are now listed and summarized:

1. Improving light reactions. The implementation of an artificial, nanocrystal light-harvesting system will be able to convert a broad range of wavelengths, leading to an increase in light-harvesting efficiency. Moreover, a bio-inspired energy buffering system will buffer the increase of metabolic energy flux coming from the increased light-harvesting and thus decrease losses due to light saturation;
2. Enhancing carbon fixation through the realization of a synthetic CO_2 fixation pathway in microalgae. This pathway will convert carbon dioxide at faster rates and in a more efficient way into the 2-carbon molecule acetyl-CoA, principal precursor for lipid production;
3. Improving lipid production and integration of genetic intervention to produce advanced microalgae solar cell factories. Microalgae will be engineered to increase the flux toward lipid production. For the project two kinds of algae have been selected: *C. reinhardtii and N. oceanica* (more details in *Chapter 3*);

4. Integration and demonstration cases of the biohybrid solar-to-fuel system. Integrating all the innovations written above, the biohybrid solar-to-fuel conversion will be demonstrated in different cases (solar irradiation of north-west Europe and north Africa). It is expected to improve the solar to biomass conversion (PE) from 2.7% to ~4.5% and solar-to-fuel conversion efficiency (SFE) from 0.5% to ~2%.
5. Perform a Life Cycle Analysis (LCA) to evaluate the impact of the whole value chain for a full-scale concept, and then integrate this analysis with a Techno-Economic Assessment (TEA) and a Social-LCA (S-LCA);
6. Encourage technology adoption and awareness of solar fuel benefits among various stakeholders, including industry, regulatory agencies, investors, NGO and consumers. The project aims to boost the utilization of microalgae in Europe and African industry, scale-up SUN-PERFORM technologies and establish the regulatory approval. Furthermore, the European export potential will be promoted, and it will develop a strong base on solar fuels;
7. Lean coordination and management of the project. A lean, ISO-compliant framework for innovation and project management will guide the project's monitoring and reporting.

The SUN-PERFOM project is carried out by several partners, and each of them has a specific task to do in order to reach the objectives (more information can be found on [17]). Among the partners, POLITO and IN are in charge of performing an Integrate Sustainability Assessment *(see section 1.5 Role of LCA and TEA)*.

1.5 Role of LCA and TEA

The importance of performing this assessment relies on the fact that sustainable bioenergy production stands at the intersection of major global challenges, representing a shift toward low-carbon, renewable energy solutions capable of addressing climate change, enhancing energy security, and promoting environmental sustainability. Thus, it is crucial to assess and elevate the sustainability and viability of bioenergy production, both from the environmental and economic points of view [18].

Regarding the LCA, an environmental assessment of the full-scale concept will be analyzed, covering the production phase of the life cycle, from feedstock to products. The aim is to quantify environmental impacts, in order to identify the environmental hotspots in the value chain. Conversely, the techno-economic assessment will assess the project from a cost, and minimum selling price point of view, evaluating the economic feasibility potential. Two different scenarios (differing in the supply source of CO_2 and in the source of nutrients) will be assessed through LCA methodology, while a preliminary minimum selling price will be estimated following TEA studies in the available literature.

Importantly, the LCA and TEA harmonization will be discussed, approaching aspects of both methodologies such as system boundaries, functional units, and assumptions.

The preliminary LCA will establish a baseline for future improvements and analyses. While the preliminary LCA will include the production of biochar from the algal biomass, the main focus of the preliminary TEA literature review will focus only on the lipid production pathway, while the assessment and valorization of the co-product as highly valuable compound and biochar will be further explored in future research, considering their potential environmental and economic benefits.

2 STATE OF ART OF LIPID PRODUCTION

2.1 State of art of current production of lipids from microalgae

Microalgae and macroalgae have been widely analyzed as feedstock for biofuel production. Microalgae, as already mentioned in *Chapter 1*, are considered a valid alternative to fossil fuels to reduce the greenhouse gas emissions and minimize the effects of climate change. The advantage of growing algae on non-arable land and feeding them with saline water is unquestionable. Besides the production of oil per hectare is higher compared to oil crops [**Table** ***3***] [19].

Table 3 Oil yield in different crops from [25]

Crop	Oil yield (L/ha)	
Corn	172	
Soy bean	446	
Canola	1190	
Jatropha	1892	
Coconut	2689	
Oil palm	5950	
Microalgae	136,900	70% oil (by wt) in biomass
Microalgae	58,700	30% oil (by wt) in biomass

In addition, microalgae have an efficient carbon fixation method, since they contain about 50% carbon, and so a good ability to absorb CO_2. Moreover, they are characterized by a rapid growth rate and high oil content. The lipid content can be enhanced under the influence of different abiotic stress factors such as light intensity, temperature, pH, media composition and others. Nevertheless, the production chain involves different steps, each with several technologies and different Technology Readiness Level (TRL), and so different processing scenarios can be developed [20], [21], [22].

Although facing significant challenges, the European algae sector has grown rapidly, with the number of producers increasing by 150% in the last decade. In 2021, around 74 companies operate across Europe, but only a small fraction (3%) focuses on biofuels. [19]. Currently, production processes are still energy intensive and uneconomical, limiting so the large-scale development [20], [21], [22]. A solution of this limitation can be the production of lipids from microalgae and the valorization of the residual through another process, in order to enhance the sustainability, both from the environmental and economic point of view [21].

The SUN-PERFORM project aims to follow this pathway, thus producing lipids from microalgae biomass and valorizing the algal residual.

In this chapter a review of the pathways for the extraction of lipids from microalgae is provided. The process can be divided into cultivation, harvesting and conversion. In our case the conversion process under interest is the lipid extraction but it can also be the conversion into a biofuel.

2.1 Cultivation

Microalgae cultivation is a critical determinant of biomass yield, influenced primarily by light, temperature, nutrient supply, and pH. Cultivation systems commonly employed include open ponds (or raceway ponds), which is an open PBR system and flat-panel, tubular, column, and plastic bag photobioreactors, which are closed PBR systems [Figure 3]. The main energy demands arise from medium mixing and circulation, CO_2 and nutrient delivery, cooling water pumping, and compensation for evaporative losses, even if there are differences, especially in the energy consumption between the closed system and the open system [20].

a) b) c)

Figure 3 a) Vertical Stack tubular PBR b) Flat-Panel PBR c) open pond

Here a review of the main cultivation systems [20]:

- *Open pond*: they are simple structures, generally open tanks or natural ponds. Their operation is simple and needs less energy compared to the closed systems. Furthermore, the investments costs are lower. Nevertheless, the land covered by the plant is larger and the productivity is lower respect PBR (~ 10-15 gr/m^2/d);
- *Tubular PBR*: these systems have a larger effective light surface area, a higher biomass productivity (~ 20-25 gr/m^2/d) and the control of the culture is easier. Though the energy demand for the operation is higher, certain conditions (pH, C02, temperature) must be maintained, the layout of the plant is more complex, it is difficult to clean, and investments and maintenance costs are higher;
- *Flat-Panel*: it is a rectangular plate made of transparent or translucent material. The system is easy clean, process and operate, costs are lower respect tubular one, present a large surface area and allows a uniform mixing of culture. The energy consumption per unit output is one tenth of that of tubular PBR. On the other hand, it is difficult to process in large industrial scale;

- *Column PBR*: Can be divided into two types: bubble column and airlift column. It is characterized by high mass transfer efficiency, a uniform mixing, a lower energy consumption and simple operation, However, it presents a small volume, high investment and maintenance costs and, as the Flat panel, it is difficult to process in large industrial scale;
- *Plastic Bag PBR*: The water consumption is low and also the costs in microalgae harvesting process are low. Therefore, it has good commercial prospects. Nonetheless it requires microalgae culture to be adsorbable and there can be problems related to the plastic bag.

Among these different cultivation technologies, the most energy consuming are the tubular PBRs, followed by flat-panel PBRs, while open ponds are the less energy requiring. For example, *Jorquera et al* [23] reported that the energy consumption, only for mechanical agitation, of open ponds PBRs, tubular PBRs, and flat-panel PBRs were 9.18MJ/kg, 385.71MJ/kg, and 16.96MJ/kg respectively.

From the point of view of Technology Readiness Level (TRL) the open pond have a TRL between 6-8 while the closed system a TRL in the range 4-5 [24].

Among the several types of reactors, open raceway ponds (ORPs) and tubular photobioreactor are the most common suitable for large-scale applications. While flat-panel, are not suitable for the scale up, since maintaining temperature control and light transmission efficiency becomes more difficult at the increasing of volume [25].

2.2 Harvesting

The harvesting stage concentrates and dewaters the microalgal culture to obtain a high concentration slurry suitable for subsequent processing. Typically, the culture medium is concentrated from 0.1–2.0% to 10–30% of total suspended solids (TSS). This process generally consists of two steps: (I) concentration, which raises TSS to about 7%, and (II) dehydration, which further increases it to around 15%. Harvesting consumes a lot of energy, about 20-30% of total energy input, making energy and cost reduction a critical requirement for large-scale production. Common methods include flocculation, flotation, electrochemical treatment, filtration, centrifugation, and sedimentation, often applied in combination [20].

Here are listed with some general characteristic [20], [26]:

- *Sedimentation*: it consists of harvesting microalgae by gravity, so it happens naturally and hence the energy consumption is low. However, this implies that requires considerable amount of time, efficiency is low and depends on the size of the particle, and the water content after the process is still high;
- *Centrifugation*: it is an improvement of gravity sedimentation and exploits centrifugal force instead of gravity to separate microalgae from the culture medium. It is a fast and effective

process, which allows a high recovery, and it is suitable for large microalgae species. In addition, it is very appropriate for recovery of high-valued products. On the other side there is the risk of cell destruction and energy consumption, investment and operational costs are high, not making it the most suitable solution for large scale applications;

- *Filtration*: the culture solution passes through a filter exploiting the effects of gravity, pressure and vacuum force. Since it doesn't use chemicals, the bio-feedstock harvested has a better quality There are several advantages such as the high recovery, the process doesn't require chemicals, the energy consumption in low (except for the case of vacuum filtration), it's time-saving and the water can be recycled. However, the process is not suitable for small microalgae and it requires a pressured or vacuum environment.
- *Flotation*: the process collects biomass by producing significant amount of microbubbles. The procedure is suitable for large-scale harvesting, costs are low, and it isn't time-consuming. But it requires surfactants and so the cost for preparing microbubbles is high;
- *Flocculation*: the process is fast and simple, is suitable for large-scale harvesting, the risk of damage the cells is lower, so it is suitable for various microalgae and moreover the energy consumption is low. Otherwise, flocculants and operators necessities high costs, it is difficult to separate flocculants from culture and the culture medium that can be recycled is limited. Based on the type of flocculants used it can be chemical, physical or biological flocculation.

So, depending on the aim of the plant, one harvesting process is more suitable rather than another. *Singh et al* [26] reported criterion for deciding the optimal harvesting technique. For example, Flocculation results the best option regarding costs, while the best option concerning the processing time is the centrifugation. Instead, flocculation and flotation are suitable for large scale application thanks to the lower cost of operation, whereas centrifugation and filtration are still unsuitable for large scale application due to the significant energy requirement. Thus, based on the main criterions (costs, processing time, biomass quality, biomass quantity, species specific and toxicity), flocculation, centrifugation and filtration represent the three most widely adopted harvesting techniques. These methods can be applied individually or in combination to improve efficiency, with the specific approach determined by the algal species, the required concentration, and the quality specifications of the final product. While, regarding the TRL, the harvesting techniques explained above ranges between TRL 6-9 [24], [26].

After harvesting, raw bio-feedstock retains a high-water content, necessitating drying before further processes. Drying improves transportability, prevents microbial contamination, extends storage stability, and is essential for efficient oil extraction, as dried biomass yields significantly higher extraction rates than wet biomass. Common drying methods include rotary, spray, solar, freeze, cross-flow, vacuum, flash, incinerator, and toroidal drying. However, the significant energy demand of these processes, accounting for about 59.3–85% of the total energy input, represents a major constraint to the economic feasibility of microalgae-based biofuels. So in order to reduce energy consumption, wet bio-feedstock can directly be sent to other processes, if these allow as a high moisture content, for example production of bioethanol, biogas etc. [20].

2.3 Lipid extraction

Lipids are biomolecules soluble in organic solvents but insoluble in water and are classified as polar or non-polar according to their chemical structure and polarity. Polar lipids form the cell membrane, whereas non-polar lipids (or neutral lipids), including acyl-glycerols (mono, di and tri) and free fatty acids, serve as energy reserves. Both polar and neutral lipids are mainly composed of fatty acids (C12-C22), either saturated (no double bonds) or unsaturated (at least one double bond) [22].

The extraction of lipids from microalgae is generally performed using organic solvent, either directly or in combination with pretreatment processes aimed at disrupting the cell and thereby aid the lipid extraction. Thus, following the harvesting and drying (optional) steps which aim to increase the concentration of the microalgal biomass, lipid extraction can be performed either on dried biomass or directly from the wet concentrate. So, two pathways can be conducted: the dry route and the wet route, and in both pathways the solvents play a key role [Figure 4]. The dry route aims to reduce the energy consumption exploiting the drying step, instead the wet route, which avoids the drying process, require a cell disruption step before the extraction of lipids. [22], [27].

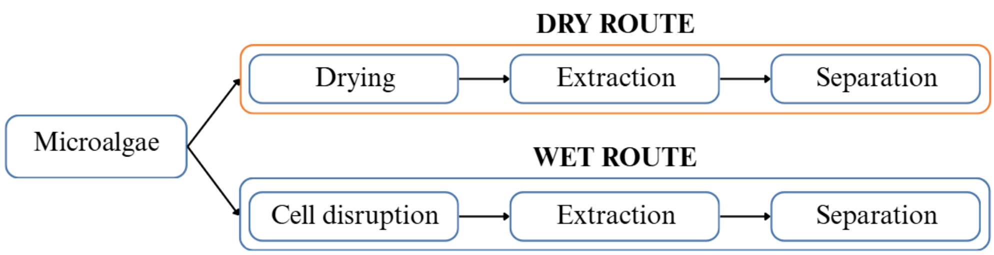


Figure 4 Pathways for lipid extraction

2.3.1 Cell disruption

Cell lysis enables the release of intracellular components such as lipids, carbohydrates, proteins and other high-valuable compounds. It can be achieved mechanically exploiting forces such as shear forces or energy transfer through waves, or non-mechanically, using solvents, enzymes, or osmotic shock. Non-mechanical methods are milder, allowing the release of products without abrupt destruction of the cells and potentially enabling the reuse of microalgae for different extraction using the same culture. Thus the cell disruption to increase the lipid extraction efficiency [27].

Mechanical lysis

This method generates force using devices such as bead milling, high pressure homogenization, screw expeller and ultrasonic. The principle is the same, the difference relies on the equipment used.

- *Bead milling*: use small glass, ceramic or steel beads that when stirred or agitated crush the microalgae cells exploiting their kinetic energy. This method presents a high disruption efficiency for a single-pass operation, and besides this, it is interesting also for its low labor intensity, high biomass throughput and operating condition control systems [27].
- *High-pressure homogenization (HPH):* this process compels cells to pass through a narrow valve where the high-pressure impact leads to the disruption of the cells. The hydrodynamic cavitation caused by the pressure drop in the valve is responsible for the cell disruption. The cavitation is defined into three steps: formation of bubbles, growth in number and collapse. When the microbubbles collapse this enforces an energy release in a high-density location resulting in cell destruction [27].
- *Ultrasonication:* this method is similar to the HPH in the formation of cavitation, but in this case is caused by high-frequency sound waves. High shear forces are induced by the propagation of shock waves generated by jet streams. An alternative to ultrasonic is the use of microwaves [27].

Non-mechanical lysis

Chemical agents/enzymes and also the application of osmotic shock are required for the non-mechanical cell disruption. The principle of these methods relies on the interaction between chemical components and the cell wall, in order to perforate the structure and enable the intracellular compounds to permeate out of the cell.

- *Acid & alkali treatment:* Exploiting the saponification process the cell wall/membrane is solubilized in order to release the intracellular components. At high temperatures (160 °C) the disruption of cells is more efficient compared to the operation at lower temperatures. However, acid and alkali have effect on the cell products and so limits its overall efficiency within a biorefinery [27].
- *Enzymatic treatment*: in this method, enzymes target the polysaccharide molecules that constitute the cell membrane (hemicellulose and saccharides), hydrolyzing their bonds and thereby degrading the structure. This process is milder/gentler respect others, moreover, operating under mild conditions the energy consumption is lower and thus also the overall costs [27].
- *Microwave irradiation:* it is a highly efficient method for lipid extraction and often is integrated with organic solvents extraction (Bligh and Dyer, Folch). It’s particularly suitable for cell with thick walls, where the use of organic solvents alone would be ineffective [27].

2.3.2 Lipid extraction method

As mentioned above, there are several methods suitable for lipid extraction, generally this process relies on the use of organic solvents which interact with lipids and protein of the cell, even if there are also other methods that don't imply the utilization of solvents [22], [27].

Solvent extraction

The conventional approach consists in direct solvent extraction. The extraction of lipids using organic solvents relies on the principle of "like dissolves like", meaning that polar solvents dissolve polar lipids, while non-polar solvents dissolve non-polar lipids. Different solvents are capable of dissolving lipids, such as acetone, benzene, chloroform, n-hexane, methanol, ethanol, butanol, and cyclohexane, among these, methanol, chloroform, and hexane have been considered as the most effective for lipid extraction. An ideal solvent should exhibit high specificity for intracellular lipids, possess a low boiling point, penetrate the cell matrix efficiently, be insoluble in water, inexpensive, volatile, and non-toxic in nature [22], [28].

The fundamental mechanism of lipid extraction by organic solvents is diffusion. Generally, a mixture of both polar and non-polar solvents, in a certain ratio, is used to ensure complete recovery of intracellular lipids. Non-polar solvent penetrates into the cytoplasm, forming complexes with neutral lipids that, driven by a concentration gradient, diffuse out of the membrane into the bulk solvent, while a fraction remains bound to polar lipids in the membrane. Polar solvents are therefore required to release these neutral lipids by disrupting hydrogen bonds, although this process also extracts polar lipids. The recovered lipids are then obtained by removing the solvents through distillation or evaporation [22].

The choice of solvents and ratios in which are employed directly affect the efficiency of the extraction. The Bligh and Dyer's method, chloroform:methanol 1:2 (v/v), and the Folch's method, chloroform:methanol 2:1 (v/v), are the most commonly employed in lab scale studies, as they provide rapid procedures with high efficiencies. These methods are effective both on the wet and dry route, reporting efficiencies up to 94.9% for Folch's method and higher than 95% for Bligh and Dyers's method. Lab-scale normally exploits batch processes that stop once the solvent is saturated. To overcome this limitation Soxhlet extraction is employed, which continuously renew the solvent through evaporation-condensation cycles. Hexane or mixture of hexane:alcohol are commonly used in Soxhlet method as they are less toxic respect to the chloroform:methanol mixture. Hexane is widely used for large scale lipid extraction due to its low cost, less toxicity, easy evaporation, and high selectivity for neutral lipids. However, the energy demand for solvent evaporation in Soxhlet method remains an important factor to consider for the overall efficiency [Figure 5] [22], [28].

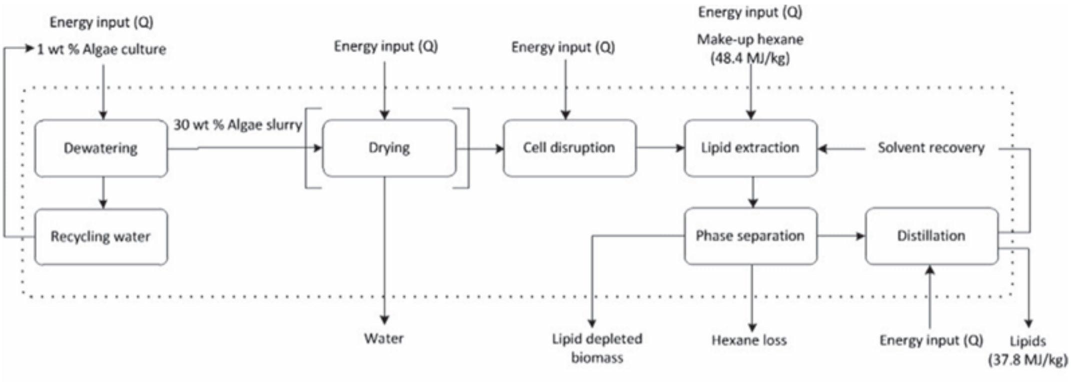


Figure 5 process scheme of hexane extraction of lipids from dry/wet microalgae from [29]

Another way to extract lipids is through Switchable Solvents. They are a subclass of ionic liquids that have emerged as promising low-energy methods for extracting lipids directly from wet algal slurries. They can change their properties in response to a trigger, generally CO_2, switching polarity (SPS), hydrophilicity (SHS), or ionic strength (SW). Classification relies on their properties thus there are switchable polarity solvents (SPS), switchable hydrophilicity solvents (SHS), and switchable water (SW). In microalgal lipid extraction, lipids dissolve in the low-polarity form of SPS, and the CO_2 trigger induces a polarity change, allowing the solvent to separate from the lipids for collection and further processing. This technique presents benefits in terms on environmental impact and energy requirement, however more studies have to be performed to assess technical feasibility [22].

Solvent free extraction

Some environmental issues, including pollution of land and aquatic environments, derive from the use of organic solvents such as chloroform, hexane, acetone and methanol. An alternative to organic solvents extraction is by mechanical means, but costs and end product damage are a considerable disadvantage. To date, there aren’t alternatives to organic solvents that can extract lipids with comparable efficiency, however the development of a 'solvent-free' extraction process could represent both an environmentally sustainable and economically advantageous approach [27].

The osmotic pressure method is one of the solvent-free extraction. The process is efficient and cost effective due to its simple setup. It is one of the simplest methods, however, currently it has only been studied at pilot scale using various freshwater microalgae. The principle of this method relies on the presence of salt in aqueous medium that creates an osmotic pressure imbalance between the cell and its surroundings. The osmotic pressure can induce either hyper-osmotic stress or hypo-osmotic stress. In hyper-osmotic conditions, higher external salt concentration drives intracellular fluids out of the cell causing shrinkage. Instead, hypo-osmotic stress, with higher intracellular salt concentration, leads to exterior fluid entering the cell bursting the cell, and so releasing the intracellular product [27].

Another method relies on the use of enzymes. The use of enzymes facilitates cell disruption under mild conditions, such as atmospheric pressure and low temperatures, but its applicability is restricted to specific microalgal strains and lipid classes. Although more cost-intensive respect other processes, this method achieves higher efficiency under mild conditions but often requires complementary mechanical disruption to break the rigid cell walls of certain species. However, a major limitation of enzymatic extraction is the need to separate lipids without compromising enzyme activity, as enzyme deactivation requires frequent replenishment and significantly increases costs [27].

A further process is the supercritical fluid extraction (SCFE). Supercritical fluids are a valid alternative to organic solvents due to their benefits in respect of health, safety and environment. They are substances present at a temperature and pressure above its critical point, where distinct liquid and gas phases no longer exist. Biomass is exposed to supercritical fluid under controlled temperature and pressure, lipids get desorbed into the fluid stream, from which they are subsequently recovered by condensation [22].

SuperCriticalCO_2 [Figure 6], characterized by a low critical temperature (31.1 °C) and moderate critical pressure (73.9 bar), is the most widely used in supercritical extraction due to its ability to recover temperature-sensitive lipid fractions without causing degradation. This method is safe, environmentally friendly, and presents low toxicity and flammability. Nevertheless, CO_2 by itself is inefficient at extracting polar compounds, hence, polar co-solvents, such as water, ethanol, or methanol and generally added to increase their recovery. The main disadvantage of this method is the high cost associated with its infrastructure and operation. While SCFE shows industrial potential and adequate extraction efficiency, further improvements in downstream purification are needed before full-scale application [22], [27], [29].

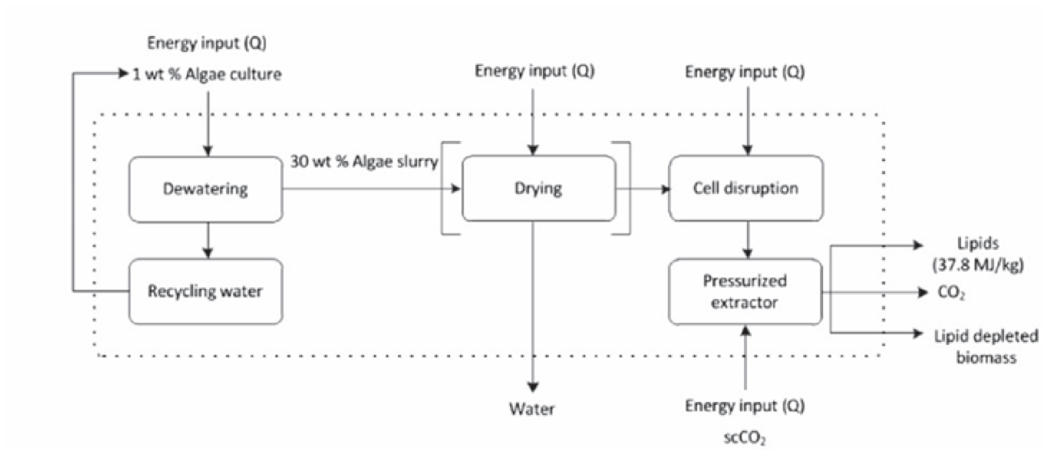


Figure 6 Process scheme of $scCO_2$ extraction of lipids from dry/wet microalgae from [29]

Instead, subcritical water is considered as a promising solvent-free method for lipid extraction. Hydrothermal liquefaction using supercritical water at 260°C reported high lipid recovery. Though, subcritical water disrupts molecular interactions in the cell membrane but further extraction with conventional solvents such hexane is still required. Regardless, this technology needs further optimization, in particular to reduce operating pressures from the current ~5 MPa [27].

The extraction of lipids, in particular methods that relies on organic solvents, presents a TRL 7-9 [24].

To date, the techniques that exploit organic solvents are the most effective in the extraction of lipids, thus, improvements in solvent-free extraction are required to bring environmental benefits and to become economically profitable.

2.4 Final considerations, challenges and future developments

Considerations regarding the profitability of the production of microalgae biomass can be performed by exploiting the Energy Consumption Ratio (ECR) (1) and the Net Energy Ratio (NER) (2).

$$ECR = \frac{E_{input}}{LHV_{biomass} * productivity} \qquad (1)$$

$$NER = \frac{E_{output}}{E_{input}} \qquad (2)$$

Many studies indicate that the net energy ratio (NER) of microalgae biomass production is below 1, particularly if cultivation is exploited in closed PBR. This makes it unprofitable in the long term. Indeed, a process is considered profitable only if NER is higher than 1. However, microalgae are rich in high-value compounds (proteins, polysaccharides, fatty acids, vitamins, pigments), which can provide profitable by-products. Their carbon sequestration capacity also creates opportunities in carbon trading [20].

In Figure 7 a review of the production to extract lipids from microalgae. As mentioned above, energy requirements, scalability, and technology readiness level hinder the definition of a unique pathway.

Improvements in cultivation and processing technologies are expected to lower energy demand in the whole production system and thus reducing also production costs. Innovation constitutes a critical factor for making microalgal production more viable

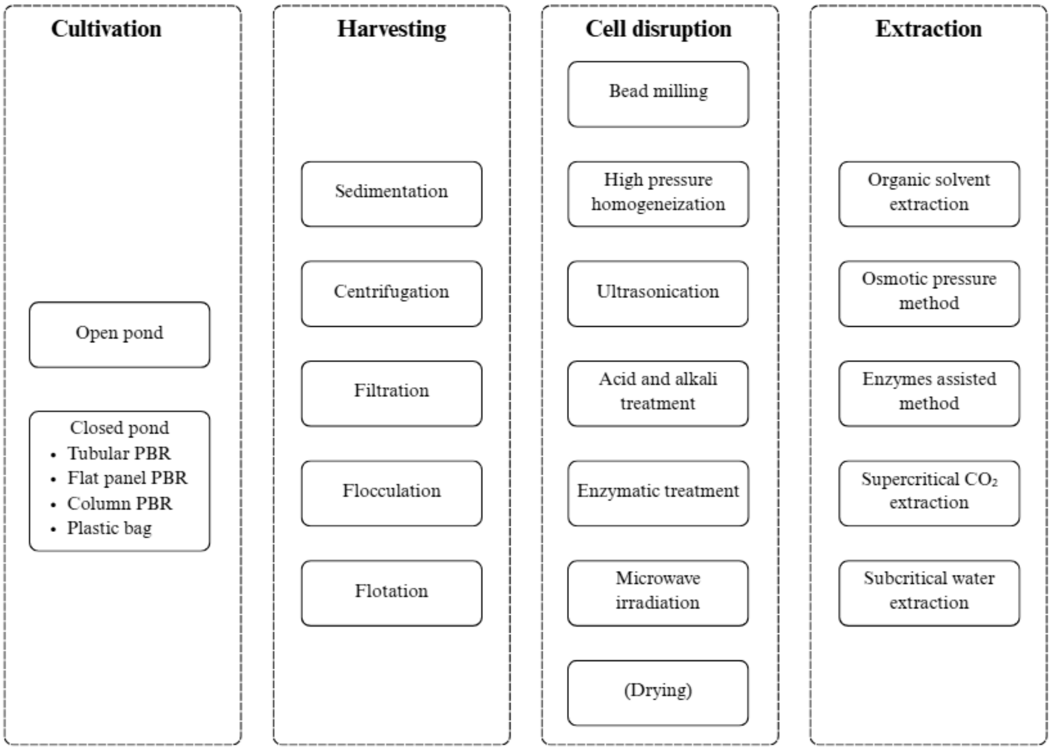


Figure 7 Summary of possible technologies for lipid production from microalgae from cultivation to lipid extraction

3 – PROCESS DESCRIPTION AND SCENARIOS

In this chapter it will be provided a description of the processes and the scenarios that will be analyzed.

In the SUN-PERFORM project to develop a solar-to-fuel technology suitable for both freshwater and saltwater, and so feasible with different locations, two different solar cell factories will be cultivated. One is based on *Chlamydomonas reinhardtii* and another on *Nannochloropsis oceanica.* This allows to expand the potential impact of the project since each species offers peculiar advantages.

Chlamydomonas reinhardtii, a freshwater green microalga. Under nitrogen deprivation, it is capable to produce high amounts of Triacylglycerols (TAGs) (40% of dry biomass) and starch. Its high versatility to genetic modification makes it a valuable model system for synthetic biology. [30], [31], [32], [33], [34], [35].

Nannochloropsis oceanica, an oleaginous marine microalga from the brown lineage, that under nitrogen starvation accumulates significant levels of TAG (50-60% of dry biomass). Moreover, its versatility to change growth conditions make it very appropriate for outdoors conditions [36].

Thus, these two microalgae strains [Figure 8] are suitable for the genetic modifications required in the project and allow to test the project by exploiting both with freshwater and saltwater.

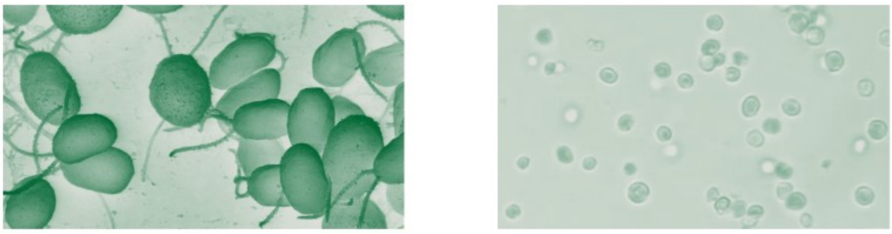


Figure 8 Chlamydonomas reinharditii (left) and Nannochloropsis oceanica (right)

Another innovation in the SUN-PERFORM is the implementation of Nanocrystal polymeric foils (NC foils) to increase the natural-light harvesting. New classes of molecular dyes and nanomaterials are capable of converting solar radiation into target wavelengths with very high efficiencies (80–99.9%) while remaining stable to photobleaching. Among them, recently developed nanocrystals (NCs) stand out as promising candidates, as they combine high energy yield with broad spectral tunability, strong photostability, and cost- and energy-efficient production [37].

After this brief discussion about the novelty of the project, a general description of the process is provided. Since the project is still in the first phase and data from partner have not been provided yet, both data and processes description are taken from literature, being this is a Preliminary Life Cycle Assessment,

An industrial-scale photobioreactor plant has been taken as reference for the first two steps. As multiple PBR technologies exist, the Vertical Stack tubular PBR (VSt-PBR) and the Flat-Panel PBR were considered in this work.

First of all, a general description of the VSt-PBR plant is provided. The plant's core consists of several PBR (VSt-PBR [Figure 9 Vertical Stack tubular PBR) modules used for the cultivation of the microalgae. The plant has an annual operativity of 300 days. The facility includes (I) a polyethylene (PE) film that hosts PBRs and auxiliaries, heat exchangers and pneumatic pumps, (II) an open shed with water treatment units, compressor, storage vessel for aeration and pressurized cylinders for the supply of CO_2, (III) a concrete building with control office, inoculum section and centrifuge. Through a Reverse Osmosis (RO), tap water needed for operating the Vst-PBR is demineralized and used for cultivation and maintenance operations of periodic cleaning and sterilization. In the cleaning and sterilization phase, which is operated with an average frequency of five times per year for each PBR module, impurities and substances that may affect PBR efficiency are removed. It is performed in four steps (I) cleansing and disinfection, with sodium troclosene, (II) rinsing with demineralized water, (III) cleaning and disinfection with citric acid, (IV) final rinsing. The RO retentate, together with the spent aqueous streams, is disposed of through the sewer system [38].

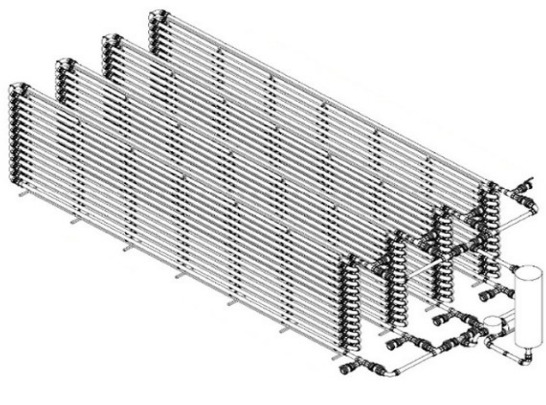


Figure 9 Vertical Stack tubular PBR

Water required undergoes Reverse Osmosis (RO) and pretreatments to remove salts, impurities and/or other unwanted substances that can interfere with the cultivation. Fresh medium is recirculated in a sterilization tank equipped with UV treatment to reduce contamination risks. Nutrients are supplied as fertilizer dissolved in water or through both fertilizers and wastewater (see *Scenario A and Scenario B*). CO_2 is supplied for photosynthesis and pH control, while aeration, mixing, and suspension circulation in the VSt-PBRs are driven by an air compressor and pneumatic pumps. Temperature in PBR is regulated within 21–27 °C to maintain the optimal growth condition by an industrial heat pump coupled along with tube bundle heat exchangers, allowing heating and cooling according to seasonal conditions [38].

The VSt-PBR plant considered in this study is composed of four VSt-PBR, for a total volume of 40.4 m^3. In the photobioreactor a semi-batch regime is applied, targeting a concentration of 2 g_{DW}/L, with an average growth rate of ~0.15 g_{DW}/L/d. The inoculum process ends in six 90 L airlift PBRs. 500 L inoculates the VSt-PBR loop, 40 L is kept for reinoculation. Cultivation proceeds via sequential dilution and batch growth (~20 days), then steady batch operation (~6 days per batch, ~32 batches/year/module). Overall, the system operates at 70% capacity. Photobioreactor are built from transparent PMMA (polymethyl methalcrylate) [38].

As mentioned, both VSt-PBR and Flat Panel were considered for this work, indeed, from literature emerges that tubular PBR are very energy intensive [20], [38], [39], an alternative to consume less energy could therefore be the utilization of another technology for cultivation, the flat-plate PBRs.

Thus, a brief description of Flat-plate technology from a real plant is provided [40].

The Green Wall Panel (GWP) is a Flat Panel photobioreactor with a disposable culture chamber, first patented in 2004. The improved version, GWP-II (in this work it will be referred to as FP-II, and Green Wall Panel as FP, to not create confusion with global Warming Potential), features a lighter stainless-steel frame supported by vertical uprights and a wooden base, reducing both embodied energy and cost. The culture chamber, made of flexible PAR (Photosynthetically Active Radiation) - transparent LDPE film, holds about 31.5 L of culture per meter with a 4.5 cm light path. Mixing and gas exchange are achieved by bubbling air or CO_2 through a perforated pipe at the bottom, while temperature control is provided by an internal serpentine or external heat exchanger. The FP combines low construction costs, easy operation, flexibility, and scalability, although it still presents relatively high energy requirements for mixing and cooling. The plant from which data used in this work was collected presents eight identical FP-II modules placed in parallel. The total area occupied by the plant is 10000 m^2 with an annual productivity of 36 tonnes of dry algae per hectare [40].

Infrastructure and *Cleaning and sanitization* are included in the analysis for both the PBRs. A scheme with flows is provided [Figure 10].

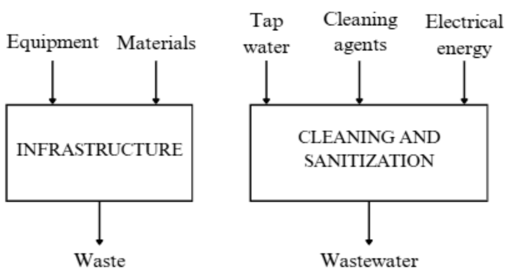


Figure 10 Infrastructure and Cleaning and sanitization scheme with main flows

For both PBRs technologies, a 2 g_{DW}/L concentration of algae is assumed at PBR exit. Therefore, the cultivation phase in the photobioreactor is followed by the harvesting phase. Here, the algal suspension produced in the PBR at 2 g_{DW}/L is sent to a dewatering step in which a highly concentrated suspension of up to 200 g_{DW}/L is reached. This is achieved through centrifugation and, the exhaust culture medium is discharged to the sewer without recirculation [38], [40].

After dewatering phase and before the hexane extraction, the algal suspension is sent to bead milling homogenization, where the algal cells are broken to perform a better lipid extraction in the following step. It is a mechanical process so an energy input is required [28].

After cell disruption, the algal suspension is sent to the lipid extraction through hexane. The hexane in the process is considered to be 1.2 times the algae-lipid-water slurry. The efficiency of lipids harvest is about 95%. The lipids and the hexane phase are sent to a distillation step in which the lipids are separated from the solvent. A large share of the latter is recirculated back in the extraction phase. However, there is a limited loss of hexane in this step, so a make-up solvent is required in the extraction step [28], [41].

Lipids, the primary target product of the SUN-PERFOM project, are recovered during the distillation step, whereas the residual algal biomass remaining after hexane extraction is directed to a valorization process. To valorize the co-product, a Hydrothermal Carbonization (HTC) is performed. To produce and extract biochar, aqueous phase and gas, energy and heat are required to operate at 180 °C and 1.3 MPa [42]. Biochar, which is a co-product of the analysis, can be used in different ways. It can be employed as fuel feedstock or in agriculture thanks to its microporous structure, improving soil quality and accelerating carbon sequestration. The characteristic of biochar depends on process conditions (residence time, heating rate, temperature, particle size etc). Biochar is largely exploited in the soil amendment process, remediation, waste and wastewater treatment, biomedical applications, sensors, energy storage, carbon capture, bio-energy, bio-composite etc [43]. Instead, aqueous phase and gas, which still presents an energy content, low, that can be exploited internally to produce heat in the HTC step.

In this preliminary study, two scenarios are analyzed [**Table *4*** Scenarios analyzed in the preliminary LCA]: Scenario A and Scenario B. Improvements and innovations of the SUN-PERFORM project are not included, in this way this analysis establish a baseline of the process. In both cases, *C. reinhardtii* is the algae strain examined, reporting, under normal conditions, 25% lipid, 45% protein, 8.80% carbohydrate on dry base weight [35]. In the two scenarios, the main phases of the process are the same (cultivation, harvesting, cell disruption, lipid extraction, HTL), the differences regard the source of CO_2, and the nutrients. Both scenarios perform the study in a location placed in Europe, more specifically in Wageningen, Netherlands. Thus, while performing the analysis, the electricity mix considered is the Netherlands one, which is characterized by a share for electricity generation from oil, coal and Natural Gas of 45%, and from low carbon technologies (Nuclear and Renewables) of 55% [44].

Table 4 Scenarios analyzed in the preliminary LCA

Scenario	SCENARIO A	SCENARIO B
Location	Europe	Europe
CO2 source	Point source capture	DAC – Direct Air Capture
Nutrients	Wastewater + fertilizer	Fertilizer
Microalgae	*C. reinhardtii* (fresh)	*C. reinhardtii* (fresh)

3.1 Scenario A

Scenario A [Figure 11] analyzes the production of lipids exploiting as source of CO_2 a point source technology, instead nutrients are provided from municipal wastewater (WW), considering the amount of water limited to the capacity of photobioreactor, while the reaming share required is provided from fertilizer.

The concentration of nutrients in a medium municipal wastewater is reported to be 40 mg/L of N and 10 mg/L of P. In the case of strong municipal wastewater, the concentration is 85 mg/L of N and 20 mg/L of P. Other wastewater/effluent can reach higher values such as the effluent of dairy/food processing, the digested starch and the biomethanated distillery, in which concentration varies between 240-4000 mg/L of N and 22-2000 mg/L of P [45]. As mentioned above, the remaining share of nutrients necessary is provided by fertilizer, ammonium nitrate and inorganic phosphorus fertilizer.

The point source capture is the simplest CO_2 capture method, removing carbon dioxide from flue gases that typically exit at atmospheric pressure with low concentration of CO_2. This technique can be applied to already existing power plants without major modifications, making retrofitting easier. Because of the low CO_2 concentration (~10%), flue gas offers little driving force for capture, requiring so large equipment and high capital costs. Moreover, contaminants such as SOx, NOx, and fly ash further increase the cost of separation with current technologies, leading to high CAPEX and OPEX and so, remarking the need for more cost-effective solutions. CO_2 separation from flue gas is challenging, as equipment must withstand high temperatures and the gas requires cleaning before capture, both of which increase process costs. The main technologies that can be exploited are absorption, adsorption, clathrate hydrate process, membrane technology, and calcium looping carbon capture [46].

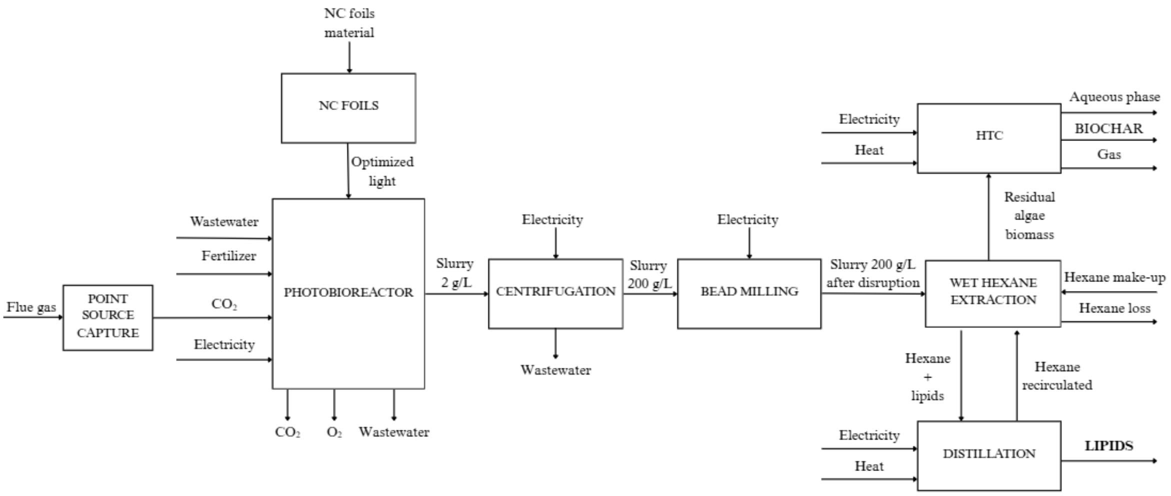


Figure 11 Scenario A: scheme with processes and flows

3.2 Scenario B

Scenario B [**Errore. L'origine riferimento non è stata trovata.**] instead studies the production of lipids utilizing Direct Air Capture (DAC) technology for CO_2 and fertilizers to provide nutrients.

CO_2 is provided by DAC technology. DAC consists in capturing directly from the atmosphere the carbon dioxide, circulating air through regenerative filters. It is generally characterized by high CO_2 removal efficiency and so can achieve net-zero or even net-negative emissions. Nevertheless, the concentration of carbon dioxide in air is low, making the process energy intensive [47]. The one considered is the adsorption technology, based on Temperature Vacuum Swing (TVS). A filter composed of amine-based active material and porous support is crossed by ambient air. Under vacuum and elevated temperature occurs desorption. Currently adsorption technology, is reported to have TRL 7 [47], so further improvements are required to make it fully commercial developed.

Nutrients instead are provided as fertilizer, in particular ammonium nitrate and inorganic phosphorus fertilizer.

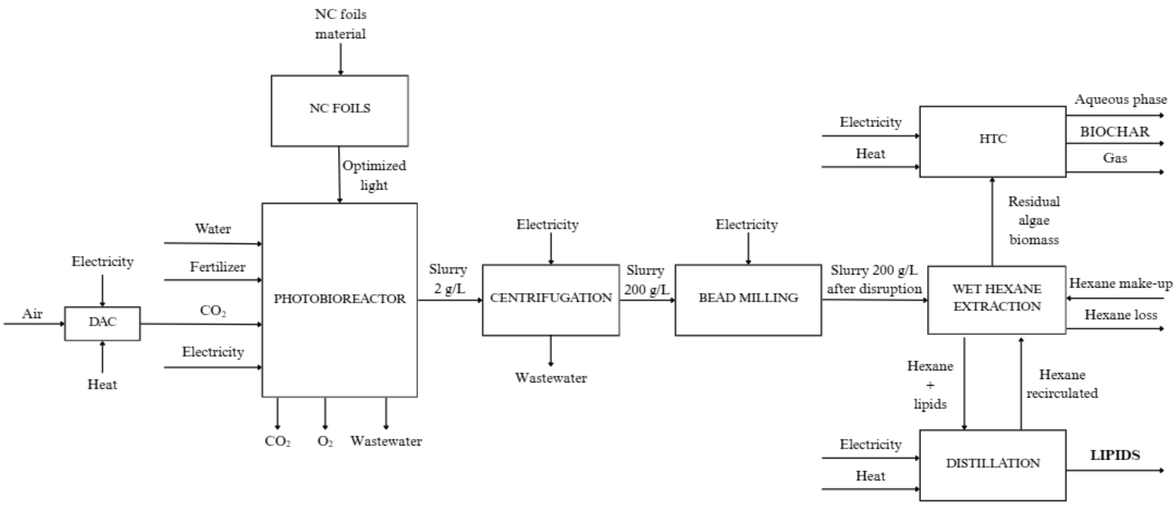


Figure 12 Scenario B: scheme with processes and flows

4 GENERAL DESCRIPTION OF LCA AND TEA

4.1 Life Cycle Assessment

In ISO 14040 LCA is defined as the "compilation and evaluation of the inputs, outputs and potential environmental impacts of a product system throughout its life cycle". Thus, LCA is a tool for the analysis of the environmental burden of products at all stages in their life cycle – from the extraction of resources, through the production of materials, product parts and the product itself, and the use of the product to the management after it is discarded, either by reuse, recycling or final disposal (defined as "cradle-to-grave analysis). The total system of unit processes involved in the life cycle of a product is called the "product system" [48].

Most important, the LCA analysis involves a "holistic" approach, bringing the environmental impacts into one consistent framework, wherever and whenever these impacts have occurred, or will occur. This approach offers core opportunities for indirect environmental management along the whole chain related to a product.

For the reasons explained above, the main applications of LCA are:

- To analyze the origins of problems related to a specific product;
- To compare improvement variants of a product;
- To design new products;
- To choose between comparable products.

So, LCA plays a useful role both in public and private environmental management in relation to products. Another application concerns eco-labeling, a tool that allows consumers to compare products. Positive examples are the "EU-Ecolabel", the "Nordic Swan Ecolabel" and the "Blue angel".

Apart from direct product applications, LCA can be used in a wider sense, for example can be applied to complex business strategies or government policies relating to consumption and lifestyle choices in various sectors of society.

Thus, LCA is a powerful tool and its "holistic" nature, which is its main characteristic and strength, at the same time is its limitation. A complete analysis of the whole life cycle can be achieved only by simplifying other aspects.

Some other limitations are related to the fact that LCA cannot address localized impacts, so it doesn't provide the framework for a local risk assessment study, so identifying which impacts can be expected due to the functioning of a facility in a certain region. The same is true for the time aspect. Indeed, LCA is steady-state, rather than a dynamic methodology. However, technological developments are increasingly taken into account in more detailed LCA studies.

Besides, LCA is a tool based on linear modeling, so all processes are considered as linear, both in the economy and in the environment. Nevertheless, LCA focuses only on the environmental aspects of the product, and does not deal with economic, social and other characteristics.

A further limitation can be the availability of data. Databases are being developed in various countries, and the format for database is being standardized. However, in practice, data are usually obsolete, incomparable, or of unknown quality. A section related to quality of data will be explored further in a subsequent section.

Finally, LCA must be considered as an analytical tool, and in this way provide information support. It cannot replace the decision-making process itself. Hence, a decision is made based on an LCA study and other evidence, and not that an LCA study has proved that a decision must be made [48].

4.1.1 REGULATORY REFERENCES AND METHODOLOGY

When discussing LCA it is important to distinguish between methodology and regulations. Indeed, LCA is still too subjected to variations that depend on who executes the analysis. For this reason, different organizations are working towards a more harmonized approach. Currently, there are the ISO standards that provide guidelines and principles to perform the LCA, considering the whole life cycle and taking into account different environmental impact categories. On the other hand, there are regulations, such as the REDII, that do not explain a methodology but set rules, boundaries and limits for specific sectors. Thus, these regulatory frameworks exploit the LCA logic but simplify and restrain it to serve policy and compliance objectives.

RED II – Renawable Energy Directive

The European Union (EU) has developed an extensive legislative framework aimed at promoting the adoption of biofuels in the transport sector, with the main goals of reducing greenhouse gas (GHG) emissions, guaranteeing sustainability, and improving energy security [49]. In *Table 5* the EU's biofuel policies and their evolution with the main targets are reported [50].

Table 5 EU's biofuel policies, their evolution and main targets

EU legislation	Targets
Directive 2003/30/EC (Biofuels)	➢ 5.75 %biofuels in transport by 2010
Directive 2009/28/EC (RED)	➢ 10% target for RES in transport by 2020
Directive 2009/30/EC (FQD)	➢ 6% reduction of GHG emissions from energy supplied in transport; ➢ Regulations on biofuels blends for gasoline and diesel
Directive 2015/1513 EU (iLUC)	➢ A cap of 7% on the contribution of biofuels produced from "food" crops

Directive 2018/2001 (REDII)	➢ 14% target for RES in transport in 2030 (32% in total); ➢ 3.5% sub-target for advanced biofuels in 2030
Revision of REDII, Fit for 55 package (2021)	➢ 13% reduction in GHG intensity or 29% RES share in the final energy consumption in transport in 2030; ➢ Objective of 40% of RES total
Directive 2023/2413 (REDIII amending REDII)	➢ 14.5% GHG reduction target in transport in 2030 or share of RES at least 29% (42.5% in total, with a 2.5% top-up potential); ➢ 5.5% sub-target for advanced biofuels in 2030 (included 1% minimum of RFNBO)

To support policy implementation, RED II Annex V and Annex VI provides pre-calculated typical and default values for biofuels that operators can use for certification purposes. The certification process is managed through voluntary and national schemes approved by the European Commission, ensuring adherence to the requirements of the RED and its implementing legislation. [49].

RED II employs a simplified technology to calculate biofuel carbon intensities, although it doesn't aim to be ISO compliant. The methodology is attributional, applying a cradle-to-use boundary for the analysis. This necessity the calculation of the three mainly GHG emissions: carbon dioxide, methane and nitrous oxide, and only considers direct emissions, applying the GWP over a 100-year time horizon. The evaluation encompasses the estimation of emissions from different stages, including cultivation, processing, transportation and combustion. To consider multi-functionality, the RED II methodology mainly relies on energy allocation, while residues and wastes are assigned with zero GHG emissions until the point of collection. The analysis adopts a static approach, annualizing dynamic emissions, such as those from land-use change, over a 20-year period, while infrastructures and end-of-life processes are excluded from the evaluation [49].

The formula (1) to estimate GHG emissions from the production and use of biofuels considers also saving and credits. It is now reported from RED II Annex V [11] with an explanation of the terms:

$$E = e_{ec} + e_l + e_p + e_{td} + e_u - e_{sca} - e_{ccs} - e_{ccr} \quad (1)$$

E = total emissions from the use of the fuel

e_{ec} = emissions from the extraction or cultivation of raw materials

e_l = annualized emissions from carbon stock changes caused by land- use change

e_p =emissions from processing

e_{td} =emissions from transport and distribution

e_u =emissions from the fuel in use

e_{sca} =emission savings from soil carbon accumulation via improved agricultural management

e_{ccs} =emission savings from CO2 capture and geological storage

e_{ccr} =emission savings from CO2 capture and replacement.

To align biofuels with climate targets, RED II/III establishes minimum GHG saving thresholds, calculated against a fossil fuel comparator for transport. Annex V provides typical and default CO_2-equivalent values, based on the JRC methodology for biofuels, bioliquids, and solid and gaseous fuels. GHG emissions are expressed in grams of CO_2 equivalent per megajoule of fuel (gCO_2eq/MJ) across all supply chain stages, allowing a comprehensive assessment of biofuel pathway carbon intensity. The reported values cover different biofuels, including biodiesel, bioethanol, and biogas/biomethane, from various feedstocks [49].

The methodology offers a robust and harmonized framework for assessing biofuel carbon footprints, allowing comparison of different pathways and highlighting those with the highest contribution to both energy and climate goals. [49]

ISO

An important role is played by the ISO standardization process. An important aim is to make these assumptions and choices as transparent as possible; this is also an important element for embedding LCA in procedures.

ISO (the International Organization for Standardization) is a world-wide private organization, including national bodies from both industrialized and developing countries, which aims to standardize a wide range of products and activities.

The 14000 series is a set of norms, standards, and tools for environmental management:

- ISO 1400X : Environmental management system;
- ISO 1402X: Environmental labels;
- ISO 1403X: Environmental performance;
- ISO 1404X: Life cycle assessment;
- ISO 1405X: Environmental management;
- ISO 1406X: Verification and validation of greenhouse gas declarations;

Relating the LCA, the standards we concern about are the ISO 14040:2006 [51] and the ISO 14044:2006 [52] that replaced the four original ones (ISO 14040-3) [53].

The development of the international standards for LCA (ISO 14040:1997, ISO 14041:1999, ISO 14042:2000, ISO 14043:2000) was a fundamental step to consolidate procedure and methods for the analysis and to avoid arbitrariness while performing an LCA analysis. Their contribution was crucial for the acceptance of LCA by stakeholders and international community. Thanks to the increasing number of LCA analysis, there was the need to improve the standards. For this reason, the original four have been revised, cancelled and replaced and two new standards introduced. The content has remained approximately the same but errors and inconsistencies were removed, and readability was improved [53], [54].

Thus, the ISO 14040:2006 provides a description of LCA principle and frameworks that is readable and accessible for a broader audience, while ISO 14044:2006 provides guidelines and requirements, in accordance with the principles in ISO 14040, and it is aimed for practitioners of LCA [54]. Thus, they are highly linked together with one purpose and are now considered one of the most important standards for the LCA analysis [53].

While performing an LCA, it is important to remember the main principles of the methodology which are: life cycle perspective, environmental focus, relative approach and functional unit, iterative approach, transparency, comprehensiveness and priority of scientific approach. These elements are essential and should be used as guidance for decision relating to both the planning and the conducting of an LCA analysis [54].

4.1.2 Four PHASES

The New International Standard for LCA (ISO 14040 and ISO 14044) outline the steps to follow to perform an LCA analysis. ISO 14040 describes them in a general way, instead ISO 14044 provides guidelines and more details.

The following description of the four phases of the LCA methodologies is taken from "the "Handbook on Life Cycle Assessment" [48].

The working method for LCA is structured along a framework that has become the subject of world-wide consensus and that forms the basis of a number of ISO standards. This framework divides the entire LCA procedure into four distinct phases [Figure 13 Life Cycle Assessment phases :

- Goal and scope definition;
- Life Cycle Inventory;
- Impact Assessment;
- Interpretation.

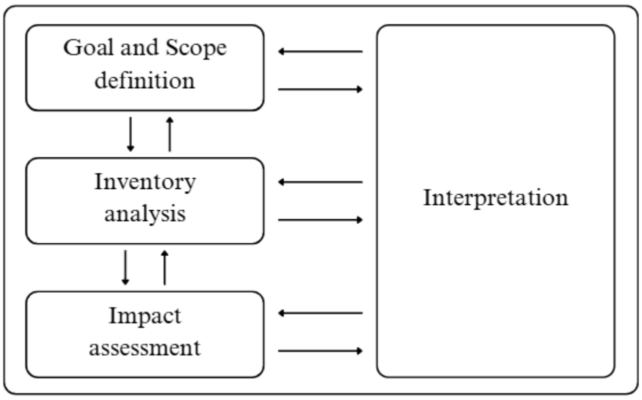


Figure 13 Life Cycle Assessment phases

The final report of the LCA is a crucial issue. A technically excellent LCA without a transparent and unambiguous report will be of extremely limited value. The baseline quality of an LCA can only be guaranteed if all steps undertaken are clearly reported. Are now listed some general principles for reporting LCA:

- all issues should be reported;
- all issues should be reported in a transparent way;
- all issues should be reported explicitly.

1) GOAL AND SCOPE DEFINITION

In this phase are made the initial choices that will determine the working plan of the entire LCA. The goal of the study is formulated in terms of the exact question, target audience and intended application. Instead, the scope of the study is defined in terms of geographical, temporal and technological boundaries, and the level of sophistication of the study in relation to its goal. Then it is defined the functional unit on which the study will rely on. The functional unit describes the primary function(s) fulfilled by a product system and indicates how much of this function is to be considered in the intended LCA study.

The results of the Goal and scope definition phase consist of a clear specification of the goal of the analysis, the functional unit and the boundaries of the study. Moreover, the scope of the study will guide further choices in subsequent phases. These results will be the input for the next phase, the Inventory analysis.

2) INVENTORY ANALYSIS

The inventory analysis is the phase in which the product system (or product systems if there is more than one alternative) is defined. The definition includes setting the system boundaries, designing the flow diagrams with unit processes, collecting the data for each of these processes, performing allocation steps for multifunctional processes and completing the final calculations. The main result is an inventory table listing the quantifies inputs and outputs to the environment associated with the functional unit.

Whenever system is analyzed, system boundaries are needed to separate the system from the rest of the world. In an LCA Inventory analysis three types of boundaries can be distinguished:

- the boundary between the product system and the environment system;
- the boundary between processes that are relevant and irrelevant to the product system (cut-off);
- the boundary between the product system under consideration and other product systems (allocation).

One of the central objectives of the Inventory phase is the collection of process data, which often involves large quantities of data in electronic form, retrieved in part from dataset set up by others. To guarantee that these data are consistent and comparable, a standard data format must be developed. Each data category should be clearly defined and assigned a specific place, accompanied by a general description to facilitate and guide data entry and retrieval.

For each LCA model, the data's quality have a major impact on the results, and proper evaluation of data quality is therefore an important step in every LCA.

Theoretically, an LCA should track the whole life cycle, and so all the processes of a given product. However, it is not possible since the large amount of flow that should be considered, so a number of flow must be either roughly estimated or cut off and then ignored. Cut-off is fundamental due to a lack of data, in combination with scarcity of time and money.

Most industrial processes are multifunctional. This means that they produce more than a single product and raw material inputs generally include intermediate or discarded products. LCA practitioners face the challenge that the product system under analysis provides more functions than the one defined by the functional unit of interest. Therefore, it is necessary to make an appropriate decision on how the economic flows and environmental impacts of the systems should be allocated to the functional unit produces by the system. Decisions on the specifics of allocation will depend on the system boundaries previously defined, since these boundaries determine which inputs and outputs are relevant to the function of interest. An appropriate allocation is thus required to distribute the inputs and the outputs of all relevant processes to the appropriate product systems.

Allocation can be performed on three different bases depending on the context. Thus, allocation can be done on mass basis, to focus on how much mass of input is required for a co-product. Energy bases are preferred if physic characteristics of co-products allow their description as energy (example the co production of electricity and heat). Last, allocation on an economic basis if it has more impact to consider the economic value of the co-product. Anyway, the allocation methodology must be specified in a clear way before the analysis.

The principal result of the Inventory analysis is the inventory table, which is the starting point of the next phase.

3) IMPACT ASSESSMENT

In this phase the set of results of the Inventory analysis – mainly the inventory table – is additionally processed and interpreted in terms of environmental impacts. For this purpose, a list of impact categories is established, and models for relating the environmental interventions to appropriate category indicators for these impact categories are defined. The results are evaluated in the characterization step and, an optional normalization step, allow to indicate the share of the modeled results in a worldwide or regional total. At the end, the category indicator results can be grouped and weighted to include societal preferences, also in this case this is an optional step.

Hence, in the Impact assessment step, the results from the Inventory analysis are translated into contribution to relevant impact categories. In this regard, relevant impact categories must be identified. A list of impact categories has been established, differentiating between 'baseline', 'study-specific' and 'other' impact categories. Thus, in this step, practitioners have to select impact categories relevant to the goal of the study. Frequently, "baseline" impact categories are included in almost every LCA study. "Study-specific" impact categories are included depending on the goal and scope of the study and whether appropriate data are available. "Other" impact categories instead require further elaboration before they can be used I LCA studies, with research still in progress. Each impact category selected must be justified in relation to the goal and scope of the study.

Here some impact categories are listed with their meaning:

- *Depletion of abiotic resources*: "abiotic resources" are natural resources (including energy resources) such as crude oil, iron ore, wind energy, which are regarded as non-living.
- *Climate change*: it is defined as the impact of human emissions on the radiative forcing of the atmosphere. This can cause adverse impacts on ecosystem health, human health and material welfare. Most of these emissions intensify radiative forces, leading to an increase in temperature of the earth's surface. This is also referred as 'greenhouse effect'.
- *Human toxicity*: it regards the impacts of toxic substances present in the environment on human health.
- *Ecotoxicity*: it covers the impacts of toxic substances on aquatic, terrestrial and sediment ecosystems.

- *Eutrophication*: it refers to all potential impacts of inordinate levels of macronutrients, which the principal are nitrogen (N) and phosphorus (P). An excessive level of nutrients may lead to an undesirable shift in species composition and elevated biomass production in both aquatic and terrestrial ecosystems.
- *Acidification*: The major acidifying pollutants are SO_2, NO_x, NO_x and cause different impacts on soil, groundwater, surface waters, biological organisms, ecosystems and materials (buildings).
- *Loss of biodiversity:* effects on biodiversity resulting from interventions such as harvesting biotic resources, or the destruction or alteration of land.
- *Stratospheric ozone*: it refers to the thinning of stratospheric ozone layer due to anthropogenic emissions. This causes a significant fraction of solar UV-B radiation to reach the earth's surface, with potentially harmful impacts on human health, animal health, terrestrial and aquatic ecosystems, biochemical cycles and materials.
- *Land competition*: Concern with the loss of land as a resource, in the sense of being temporarily unavailable. The areas of protection are natural resources and man-made environment.

During the classification phase, for each impact is assigned the kind of emission that causes it. The same emission can lead to more impacts, in that case, if possible, each portion is distributed between the different impacts, if not possible the emission is attributed for each impact.

Then, impacts are evaluated using the characterization factors. So, depending also on the characterization method chosen, in which the characterization factors are defined, impacts are quantified in terms of a common unit category.

Two optional steps can be performed. The normalization step is defined as the calculation of the magnitude of indicator results relative to reference information. The reference information may pertain to a given community, person or the system, over a given period of time. Other reference information can be chosen such as a future target situation. The main aim of normalizing the category indicator is to obtain a clearer understanding of the relative importance and magnitude of these results for each product system. Moreover, normalization can also be used to check inconsistencies, to provide and convey information about the relative importance of the category indicator results and prepare for additional processes like weighting or interpretation.

The weighting step is optional. Here the normalized indicator results for each impact category are assigned numerical factors based on their relative importance, multiplied by these factors and possibly aggregated.

The main results of this Impact assessment step include the environmental profile and (optional) the normalized environmental profile and the weighting profile.

4) INTERPRETATION

In this last phase the results of the analysis and all choices and assumption made during the whole analysis are evaluated in terms of soundness and robustness, and overall conclusion are outlined. The main elements of this phase involve evaluating results for consistency and completeness, analyzing them (for instance in term of robustness), and formulating conclusions and recommendation of the study.

The aim of the consistency check is to establish whether the assumptions, methods, models and data are consistent with the goal and scope of the study, both over a product's life cycle and across various options. All other analyses of results and sensitivity analyses are useless if the assumption and models used in the LCA are inconsistent with the goal and scope of the study.

The completeness check guarantees that all relevant information and data required for the Interpretation phase are accessible and complete.

The overall contribution of the various factors is calculated during the contribution analysis. Contributions generally are expressed as percentage of the total. This analysis specifies the contribution of specific environmental flows, processes or impacts to a given environmental score.

To use LCA as a decision-making tool, it is important to have information about the reliability of the results. The sensitivity step evaluates the influence of variation in process data, model choice and other variables of results. In the sensitivity analysis, these changes are voluntarily introduced to determine the robustness of the results with respect to these variations. While the uncertainty analysis uses empirical data on the uncertainty ranges of specific data to calculate the total error range of the results.

In the last step of the Interpretation phase, conclusion and recommendation are drawn for the intended audience of the study, based on the information gathered in the previous phases of the LCA and combined with the results of the previous steps of the interpretation phase.

The results of the Interpretation phase are two-fold. First, there are the results of all forms of consistency and uncertainty analysis, leading to a number of judgements relating to the quality and the robustness of the findings of the Inventory analysis and Impact assessment. Second, there is a description of the final conclusions and recommendations, for instance regarding product choice or improvements.

4.2 Techno-Economic Assessment (TEA)

The development and improvement of technologies and production chains, beyond being environmentally sustainable, assessed through an LCA, should also be economically viable, exploiting a Techno-Economic Assessment. TEA and LCA should be integrated for more comprehensive analysis.

TEA is a methodological framework for evaluating the technical and economic performance of processes, products, or services. It examines the economic implications of research, development, demonstration, and deployment while quantifying production costs and market potential [55], [56]. TEA can assess the technical feasibility of biorefineries by evaluating for example the co-production of biofuels as the main product alongside different chemicals as co-products [56]. Sensitivity analysis can be performed to find the optimal design to obtain, for example, the minimum fuel selling price.

Summarizing, the main applications of TEA include baseline price evaluation, critical profitability analysis, and comparison of alternatives with respect to production yield, capital and operating costs, and overall profitability. It also involves the technical assessment of novel process designs, estimation of potential energy demand, evaluation of feedstock switching feasibility, and analysis of the economic impacts of integrating new technologies into existing systems. Sensitivity analysis is applied to identify key parameters, while TEA, combined with optimization tools, supports the development of optimized process designs. A range of tools are employed to perform material and energy balances, estimate equipment costs, determine capital and operational expenditures, and assess profitability. Some of the most popular commercial tools are from Aspen tech [56].

The most common output of TEA are: Net Present Value (NPV), final price of product, capital and operating cost, payback period [56].

In [55] guidelines for TEA were developed in parallel to Life Cycle Assessment guidelines, so maintaining the same structure in four phases. These guidelines for TEA analysis were developed through literature analysis, workshops and revision from experts.

In this review are reported required and recommended guidelines, which are “Shall” (or “must”) rules that sets minimum requirements to achieve, and “Should” rules, which instead are recommended requirements. As LCA, TEA is subdivided into four phases [Figure 14 Phases of techno-economic assessment: goal and scope, inventory, calculation of indicators, interpretation, and reporting.

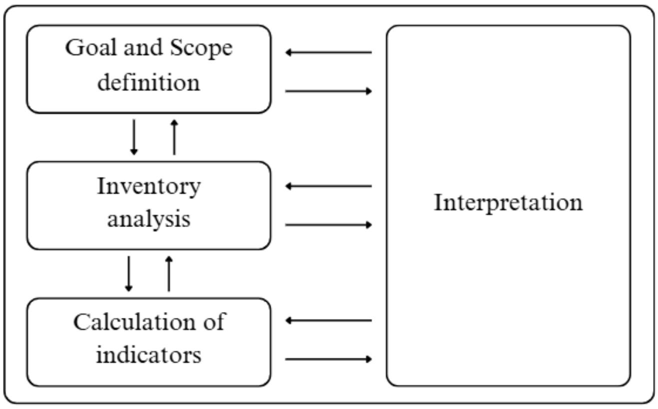


Figure 14 Phases of techno-economic assessment

1) Goal and Scope

In the first phase of TEA, practitioners define the goal of the analysis, specifying the main research questions, context, intended use, limitations and target audience. The goal of the analysis determines all subsequent parts of the assessment. It is defined by practitioners at the beginning of the study, but, with caution, adjustment can be made during the study. So, summarizing, goals of TEAs shall declare in a clear and unambiguous way its context (including comparison basis, location, time horizon, scale, and partners), its purpose (such as supporting R&D, investment, or policy decisions), the intended audience (from experts and agencies to policymakers, NGOs, media, and the public), the commissioners and authors, as well as the limitations arising from assumptions, methods, or specific use cases. Moreover, practitioners can also define scenarios and if TEA is integrated with LCA, scenario shall be the same.

Based on this goal, the scope of the study is established, determining which aspects of the product are to be evaluated and how they will be compared with competing solutions. Key tasks in this phase include identifying the product application and system under analysis, defining the functional unit and reference flow, specifying system elements and boundaries, selecting appropriate benchmark systems, assessing technology maturity, and determining the criteria and indicators to be applied. The defined scope then serves as the foundation for the subsequent inventory, calculation, and reporting phases.

2) Inventory

Following the goal and scope definition, the TEA proceeds with the inventory phase. This phase involves five interconnected steps: defining data quality requirements, identifying relevant technical processes, collecting both technical and economic data, and documenting the information obtained.

According to the assessment's goal and scope, practitioners shall define quality requirements for each data point, and data quality shall be checked and documented. In TEA analysis, data quality is improved iteratively, thus, starting with lower quality sources and progressively refining them when results are most sensitive. Practitioners should collect data aligned with the technology TRL, which indicates whether high-quality data can be obtained directly or need instead to be estimated. If practitioners cannot improve data quality up to a sufficient level, they should either adapt the goal and scope to the available data or, if this is not feasible, terminate the study.

Data collection must align with the defined requirements, methods, indicators, and assessment goal. Since TEA aims to improve both the overall product system and its individual components, data for each identified system element should be collected and documented, with the level of detail reflecting the corresponding processes. Economic data includes equipment costs, input and output prices and relevant market information. Harmonization should be maintained when collecting data from different sources, which means keeping uniformity and aligning assumptions. If possible, both technical and economic data shall be related to the functional unit and reference flow.

3) Calculation

After selecting indicators and collecting data, suitable calculation methods are applied during the calculation phase, with the resulting output forming the basis for interpretation. Best practices for calculation and economic indicators are now provided.

Practitioners shall organize separately indicator calculations and inventory but keep them linked to allow updates when inventory data change. All indicators, equations, relevant inputs and results for both individual system elements and product systems shall be listed in SI units or include a unit definition, and calculation derived shall be organized transparently.

Common economic indicators include investment costs (CAPEX), operational costs (OPEX) and profitability indicators.

- CAPEX – Capital expenditure: it includes the initial cost of designing, constructing, installing and commissioning a plant, as well as any modifications required to prepare the site. It is a key indicator for the investment criterion in TEA. It can be considered directly or used to evaluate other indicators such as Cost of Goods Manufactured (COGM) and Cost of Goods Sold (COGS).

- OPEX – Operational expenditure: it includes all production costs, encompassing both variable (direct) cost (i.e., raw materials and utilities), and fixed (indirect) cost (i.e., labor cost and maintenance).

- Profitability indicators: include profit, net present value, and internal rate of return, address the profitability criterion in techno-economic assessments. They incorporate revenues, costs (such as Capex and Opex), and risk, providing a measure of whether, how much, and when returns can be realized compared to alternative investments.

4) Interpretation

Interpretation occurs alongside all TEA phases, verifying that inventory data (inputs) and results (outputs) are checked for quality, consistency, completeness, and reliability with reference to the study's goal and scope. Main tasks include conducting uncertainty and sensitivity analyses, interpreting results, and performing multicriteria decision analysis. The outcomes, conclusions and identified limitations, form the basis for decisions and recommendations for future research, development, and deployment.

4.3 Integration of LCA and TEA

For a genuinely sustainable design, technology development should consider not only technical performance and economic feasibility but also potential environmental impacts. Techno-Economic Analysis (TEA) evaluates technical performance together with capital and operating costs to gauge economic viability, whereas Life Cycle Assessment (LCA) quantifies potential environmental impacts across the entire life cycle of a product system. When conducted in isolation, TEA and LCA provide only a partial view; integrating them allows systematic evaluation of technical, economic, and environmental dimensions and delivers more robust inputs for trade-off analysis and process optimization. Integration also helps align system boundaries, functional units, and key assumptions that often diverge when the methods are applied separately—an advantage that is especially important for prospective assessments of emerging technologies at low technology readiness levels (TRLs) [56].

Technology maturity strongly shapes assessment quality. Data availability and certainty evolve along the TRL scale (TRL 1–9), and early design decisions can lock in economic and environmental performance. At low TRLs, choices have high leverage, but data are scarce; at high TRLs, more data are available but the room for optimization narrows [56].

In practice, TEA is widely applied to process design in emerging sectors such as biofuels and other renewable-energy pathways. It is used to compare alternative production routes, evaluate the feasibility of co-products, and quantify energy demands of individual unit operations. Typical outputs include net present value (NPV), minimum selling price (e.g., MFSP), capital and operating expenditures, and payback period. While TEA and LCA are frequently carried out in parallel, most studies still rely on separate models, databases, and cost equations, which undermines direct comparability. Establishing a single, unified platform with shared process boundaries and standardized calculation bases would substantially strengthen the consistency and usefulness of integrated assessments [56].

Beyond comparability, integration unlocks optimization. Existing studies often optimize with respect to either cost (TEA) or impacts (LCA) alone; an integrated framework enables multi-objective optimization across both dimensions, clarifying trade-offs and helping to identify process configurations that achieve low production costs and low environmental burdens. Finally, practical tools that support ex-ante evaluation at low TRLs remain limited; developing such platforms, together with clear communication strategies for uncertainty and results tailored to different stakeholders, will be crucial to guide scalable, economically viable, and environmentally sound technology deployment [56].

Despite clear benefits, TEA–LCA integration is still emerging and faces several methodological hurdles [56]:

- Tooling gap. There is currently no widely adopted software capable of performing TEA and LCA simultaneously; researchers must reconcile results from separate tools (i.e., Aspen-based TEA vs. openLCA/SimaPro), complicating decision-making.
- Boundary and scope alignment. TEA often uses cradle-to-gate boundaries centered on the production process, whereas LCA typically spans cradle-to-grave. Misaligned system boundaries and functional units impede meaningful integration.
- Data interoperability. TEA and LCA use different input–output structures and conventions (i.e., unit-operation mass/energy balances and cost models vs. life-cycle inventories and impact categories), making translation to a common format non-trivial.
- Data scarcity and uncertainty at low TRLs. Processes in early stages lack complete, high-quality data, increasing parameter and model uncertainty in both TEA and LCA and making combined evaluations harder to trust without robust uncertainty analysis.

Thus, priority directions include (i) developing integrated TEA–LCA tools that update both economic and environmental metrics in parallel as process parameters change; (ii) embedding multi-objective optimization to explore the economic–environmental design space; and (iii) standardizing reporting and uncertainty communication so results are transparent and comparable across studies and useful to diverse stakeholders [56].

5 CASE STUDY – LCA

This preliminary LCA is performed with a methodology that follows the principles and guidelines reported in the International Standard norms ISO 14040 (2006) and ISO 14044 (2006) [51], [52]. Since it is a preliminary analysis, data are obtained from literature review, thus carrying uncertainties. The preliminary assessment was conducted on the OpenLCA 2.3.0 software, using the Ecoinvent v3.10 library as database [57], to model the product system and developing its background inventory.

5.1 Goal and Scope

The goal of this preliminary analysis is to develop a model of the process and to assess an initial baseline for the potential environmental impacts of the SUN-PERFORM project. The microalgae under analysis is the *Chlamydomonas reihnarditii* (composed of, based on dry weight: 25% lipids, 45% proteins, 8.80% carbohydrates) [58] without any bio-engineering interventions, cultivated in a VSt-PBR located in Wageningen, Netherlands. Also, for this preliminary analysis, a standard photobioreactor is considered, without accounting improvements in the productivity from NC foils; however, NC foils are included in the analysis to evaluate whether the material itself has an impact on the assessment.

Additionally, this preliminary study also aims to identify the environmental hotspots in the production of lipids from microalgae and to support future developments of the SUN-PERFORM project.

The approach adopted is a "cradle-to-gate" analysis, with a functional unit (FU) of 1 MJ from lipids. Data come from literature, bringing lots of uncertainties, in particular regarding the energy consumption (see in the *Interpretation* paragraph). The assessment include the impact of PBR infrastructure, cleaning of PBR, and the operation of the plant (cultivation, harvesting, cell disruption, extraction, waste valorization). The infrastructure of other parts of the plant beyond the PBR and centrifugation have not been considered. The transport of material is included by using background data modeled on Netherlands, European and global markets, preferring Netherlands modelling over European and global models. The lifetime of the plant is assumed to be 30 years, while the temporal boundaries of the analysis coincide with the current year, 2025. The geographical boundary is defined as the Netherlands, which in practice is represented by adopting the Dutch electricity mix in the analysis.

In Figure 15 and Figure 17 are reported the scheme of the whole process for Scenario A and Scenario B. Infrastructure and Cleaning and Sanitization are common for both scenarios, and they are reported in separate scheme [Figure 16**Errore. L'origine riferimento non è stata trovata.**Figure 16].

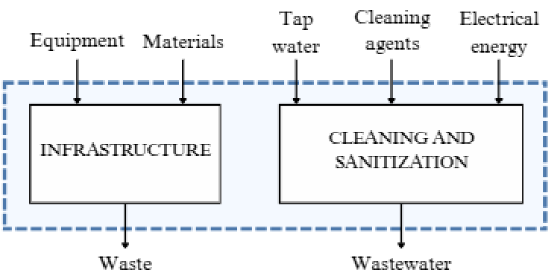


Figure 16 Infrastructure and Cleaning and Sanitization - scheme of product system with system boundaries and input/output flows

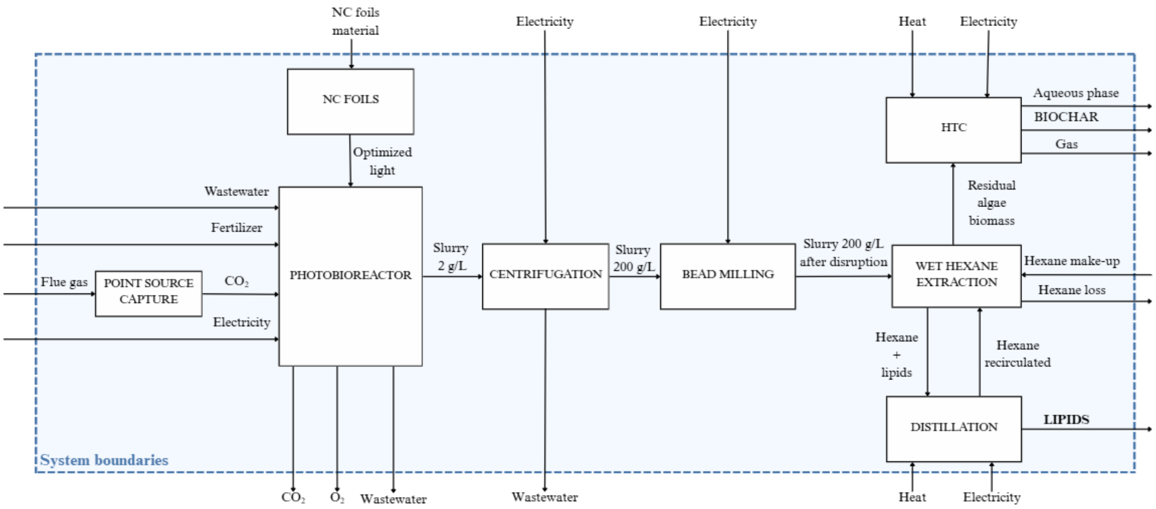


Figure 15 Scenario A - scheme of product system with system boundaries and input/output flows

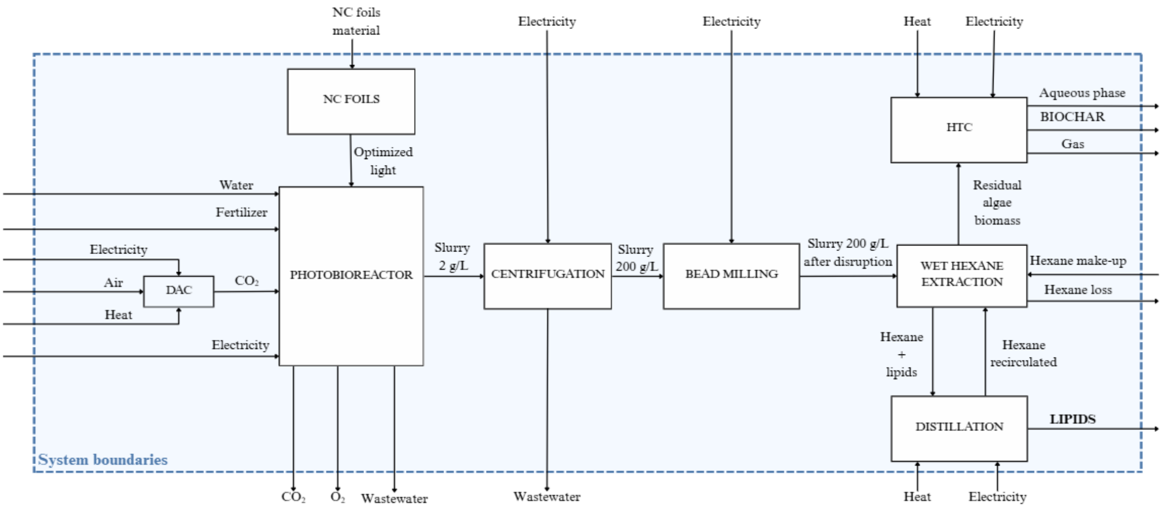


Figure 17 Scenario B - scheme of product system with system boundaries and input/output flows

In the "wet hexane extraction" step, an allocation based on energy content is performed to consider the impact of the two distinct co-products: lipids (the main product), and the algae residual biomass. The choice to perform an energy allocation derives from the REDII [11], in which it is stated that the energy allocation method is considered the most appropriate for distributing emissions among co-products, due to its simplicity, reliability, and comparability with substitution-based approaches. In allocation, the environmental burden from upstream processes is divided among the different co-products. Thus, in the energy allocation, the energy content of the two fluxes is evaluated to assign the share of impacts on each of them [Table 6 Energy allocation of the two fluxes exiting the wet hexane extraction]. Then, the algae residual is sent to a valorization step, hydrothermal carbonization, in which three co-products are obtained. In this process the allocation is again evaluated on energy basis but in this case, all the energy content is assigned to the biochar, while the aqueous phase and the gas can be used to produce heat internally.

Table 6 Energy allocation of the two fluxes exiting the wet hexane extraction phase

Flow	Mass [kg]	Energy content [MJ]	Energy allocation
Lipid	0.026	1.0	44%
Residual algae biomass	0.634	1.26	56%

5.2 Life cycle inventory

Data for the Life Cycle Inventory are obtained from a literature review. The quality of data is very important to achieve results that minimize uncertainty. However, microalgae biorefinery tends to be unlikely competitive compared to terrestrial crop technology. Indeed, due to technical and economic limitations, it has a TRL of 4-7 [24] and hence, microalgae biorefinery it is not commercially attractive [25], [59]. Due to this low commercial development, data from literature, especially in the cultivation phase, present several discrepancies.

Before performing the analysis, some considerations have been made regarding tubular PBR. Although this technology was selected in Chapter 3, literature indicates that the cultivation phase is extremely energy intensive. From literature, tubular PBRs can require up to about 29 kWh/MJ_{lipids} [38] leading to GWP100 higher than 6000 g CO_2-Eq/MJ. Indeed, as mentioned in *Chapter 2*, PBRs and in particular tubular PBR, are highly energy intensive.

For example, in *Pérez-Lòpez et al.* [39] a comparative analysis of three different reactors (Horizontal tubular PBR, vertical tubular PBR, Open Pond) is reported. Open pond technology is the one that consumes less energy, and so it leads to a lower impact; instead, both the PBRs have a larger impact. Moreover, *Pérez-Lòpez et al.* [39] suggests that, for large scale applications, ground water for cooling and waste heat from a biorefinery or power generation can be exploited to reduce energy consumption for thermoregulation. In alternative, generation of steam by the direct burning of fuel, has to be preferred with respect to electrical heating, due to a difference in efficiency (~90% and ~40% respectively). In addition, energy consumption for pumping and mixing can be reduced in a range between 26 and 249 MJ/kg_{DW}, which in our case means between 0.79 kWh/MJ_{lipids} and 7.6 kWh/MJ_{lipids}. However, tubular PBRs remain particularly energy intensive for large scale applications, therefore, a different cultivation technology was considered. Switching to another photobioreactor configuration, such as the flat-plate PBR, can significantly reduce the overall impact due to its lower energy requirements. [60].

Thus, the analysis has been performed on a Flat-plate PBR [40].

For the analysis, data from literature are considered for the infrastructure, the cleaning and sanitization phase, and the other steps (cultivation, centrifugation, bead milling, hexane extraction, distillation and hydrothermal carbonization).

To conduct the study, some assumptions were made for the analyzed scenarios. In scenario A wastewater has no impact since it is treated as a waste input with no upstream burden, following the cut-off principle. The only impacts are related to its management, in this case the transportation impact is considered, modeled as land transport with a freight from a wastewater treatment plant (Waterschap Hollandse Delta - RWZI Dokhaven) located approximately 98 km away.

The point source technology to supply CO_2 in Scenario A is modeled using the "market for carbon dioxide, for chemical industry" process, which covers impacts from industrial production, of carbon dioxide, until its provision to consumers.

In scenario B, DAC technology is modeled referring to an adsorption technology, specifically Temperature Vacuum Swing (TVS). The impact of its infrastructure is not considered as it wouldn't be significant. The sorbent material is modeled as diethanolamine and silica sand, using market datasets.

In both scenarios, fertilizers are required. To model nitrogen and phosphorus, market dataset for sodium nitrate and inorganic phosphorus fertilizer are selected.

Other assumption regarding energy inputs. Electricity is modeled as market dataset for medium voltage in Netherlands, while Heat is defined as market dataset for heat, district or industrial, natural gas in Europe without Switzerland.

An annual operation time of 240 days is considered, together with 5 complete cleaning and cultivation cycles per year [38], [40]. The infrastructural lifespan of components is considered to be 20 and 25 years for blowers and centrifuges respectively, 5 years for pumps and PVC bubbling pipes and fittings, 20 years for stainless steel for chamber framework, 10 years for timber beam years, 9 years for PVC in general piping machineries, and 1 year for LDPE film for culture chamber [40].

Cleaning and sanitization step has been modeled based on *Guerreri et al* [38], adapting the process described in *Chapter 3* to a Flat-plate PBR. Raw data from literature were elaborated and all inputs and outputs flows were reported to the functional unit (FU 1 MJ).

In Table 7, Table 8, Table 9, Table 10, Table 11 are reported the Life Cycle Inventory, all values are referred to a FU of 1 MJ from lipids.

Table 7 LCI of main processes referred to 1 MJ

Process	Input/output	Flux	Value	Unit	Reference
Photobioreactor	Input	Water			
		Scenario A	Table 8		
		Scenario B	Table 9		
	Input	CO2			
		Scenario A	275	g	
		Scenario B	Table 10		
	Input	Sodium nitrate			
		Scenario A	Table 8		
		Scenario B	Table 9		
	Input	Phosphorus fertilizer			
		Scenario A	Table 8		
		Scenario B	Table 9		
	Input	Electricity	3.0	kWh	[40]
	Input	NC foils material	2.2	g	
	Output (emission)	Oxygen	220	g	[38]
	Output (emission)	Carbon dioxide	55	g	
	Output (product)	Slurry 2g/L	55.1	kg	
	Output (waste)	Wastewater	3.85	L	
Centrifugation	Input	Slurry 2g/L	55.1	kg	[40]
	Input	Electricity	0.069	kWh	
	Output (waste)	Wastewater	54.45	L	
	Output (product)	Slurry 200g/L	0.66	kg	

Bead milling	Input	Slurry 200g/L	0.66	kg	[28]
	Input	electricity	0.076	kWh	
	Output (product)	Slurry after disruption	0.66	kg	
Wet hexane extraction	Input	Slurry after disruption	0.66	kg	[41]
	Input	Hexane make-up	0.264	g	
	Output (waste)	Hexane lost	0.264	g	
	Output (product)	Algae residual	0.634	kg	
Distillation	Input	Lipids	0.026	kg	[41]
	Input	Heat	0.046	kWh	
	Input	Electricity	0.014	kWh	
	Output (product)	Lipids	0.026	kg	
HTC	Input	Heat	0.150	kWh	[61], [62]
	Input	Electricity	0.0124	kWh	
	Input	Lipid-extracted algae	0.634	kg	
	Output (co-product)	Biochar	0.0337	kg	
	Output	Gas	0.00486	kg	
	Output	Aqueous phase	0.595	kg	

Table 8 LCI wastewater + fertilizer - Scenario A

Process	Input/output	Flux	Value	Unit	Reference
Wastewater + fertilizer	input	Wastewater	5772	L*km	[45]
	input	Sodium nitrate	0.044	kg	[40]
	input	Phosphorus fertilizer	0.0024	kg	[40]

Table 9 LCI water and fertilizer - Scenario B

Process	Input/output	Flux	Value	Unit	Reference
Water + fertilizer	Input	Water	58.9	L	[40]
	input	Sodium nitrate	0.046	kg	[40]
	input	Phosphorus fertilizer	0.003	kg	[40]

Table 10 LCI Direct Air Capture - Scenario B

Process	Input/output	Flux	Value	Unit	Reference
DAC	input	Heat	1.375	MJ	[63]
	input	Electricity	0.077	kWh	
	input	Ethanolamine	0.336	g	
	input	Silica	0.336	g	
	output	CO_2	0.275	kg	

Table 11 LCI Infrastructure and Cleaning and sanitization - common for both scenarios, A and B

Process	Input/output	Flux	Value	Unit	Reference
Infrastructure	input	Timber beam	11	g	[40]
	input	Stainless steel	11.9	g	
	input	PVC for bubbling pipe	4.6	g	
	input	LDPE	9.2	g	
	input	PVC general piping	0.61	g	
	input	Blowers	0.15	g	
	input	Pumps	0.121	g	
	input	Centrifuge	0.24	g	
Cleaning and sanitization	input	Tap water	13.8	L	[38]
	input	Sodium troclosene	0.24	g	
	input	Citric acid	92.8	g	
	input	Pumping and agitation	0.049	kWh	
	output	Wastewater	13.8	L	

5.3 Life cycle impact assessment

The life cycle impact assessment is performed using different methodologies:

- *The IPCC, Intergovernmental Panel on Climate Change, established by the United Nations, periodically publishes Assessment Reports (ARs) that provide emission metrics such as Global Warming Potential (GWP) and Global Temperature Change Potential (GTP). These metrics are applied as characterization factors (CFs) within the IPCC methodologies;*
- *ReCiPe was created in 2008 by the Dutch research institute of RIVM, Radboud University Nijmegen, Leiden University, and PRé Consultants, and later updated in 2016 to its current*

version. As both a midpoint and endpoint method, it considers three cultural perspectives: Individualist, Hierarchist, and Egalitarian, and evaluates a range of midpoint impact categories as well as the three areas of protection human health, ecosystem quality, and natural resources at the endpoint level.*

- *EF (Environmental Footprint) is a method maintained by the European Commission. It was revised from version 3.0 to version 3.1 in July 2022. Additionally, both EF v3.0 and EF v3.1 have been implemented in the EN 15804 standard, with differences in characterization factors (CFs) for biogenic CO_2.*

The impact categories analyzed in this study are climate change GWP100 (IPCC 2021), water depletion (ReCiPe 2016), acidification and eutrophication (EF v3.1). The choice of these categories was supported by a literature review of other Life Cycle Assessment analyses performed on microalgae production plants [64], [65].

As already mentioned in *Chapter 4,* a brief description of the impact categories is reported [48]:

- *Climate change (kg CO_2-Eq):* it is defined as the impact of human emissions on the radiative forcing of the atmosphere. This can cause adverse impacts on ecosystem health, human health and material welfare;
- *Water use (m^3):* It measures the relative amount of water used, accounting not only for the volume of water consumed but also regional water scarcity factors. So, areas with limited availability of water will present higher weight to water use;
- *Acidification (mol H^+-Eq):* The major acidifying pollutants are SO_2, NO_x, NH_x and cause different impacts on soil, groundwater, surface waters, biological organisms, ecosystems and materials (buildings);
- *Eutrophication - freshwater (kg P-Eq)*: it refers to all potential impacts due to an enrichment of the freshwater ecosystems caused by nutritional elements, which the principal are nitrogen (N) and phosphorus (P). An excessive level of nutrients may lead to an undesirable shift in species composition and elevated biomass production in both aquatic and terrestrial ecosystems.

5.4 Interpretation

Interpretation of results and impacts are provided partitioned into the two main flows (product, lipids, and co-product, biochar), based on the performed energy allocation. From energy allocation it resulted that lipids accounted for 44% while biochar for 56% [Table 6]. All results are reported on the basis of the Functional Unit (1 MJ of lipids).

5.4.1 SCENARIO A

The first scenario studied, as mentioned in *Chapter 3,* exploited a point source capture technology to provide CO_2 and a wastewater + fertilizer mix to supply nutrients. Table 12 presents the results for Scenario A under two frameworks: a complete framework and one where impact of *Infrastructure* and *Cleaning and Sanitization* steps are neglected.

Table 12 Scenario A - LCIA results

Scenario	Product	GWP100	WATER USE	ACIDIF.	EUTROP.
		$gCO_{2\,Eq}$/MJ	m^3/MJ	mol H^+_{Eq}/MJ	kg P_{Eq}/MJ
Scenario A	LIPIDS	396	0,0023	0,0021	0,00012
	BIOCHAR	558	0,0030	0,0027	0,00016
Scenario A w/o impacts of "infrastructure" and "cleaning and sanitization"	LIPIDS	318	0,0016	0,0017	0,00009
	BIOCHAR	460	0,0020	0,0022	0,00012

The processes' shares on each impact category analyzed are reported in Figure 18 and Figure 19.

The results show that the cultivation phase presents a significant contributor across all impact categories, ~68% (GWP100), ~54% (Water use), ~75% (Acidification) and ~34% (Eutrophication).

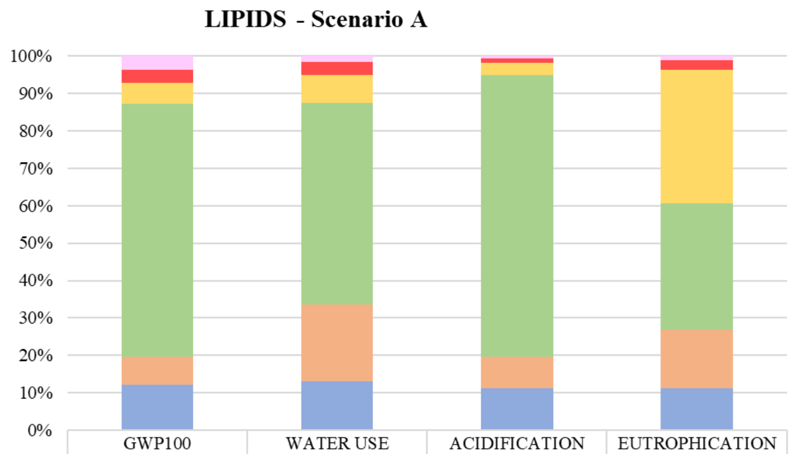
Infrastructure has an impact of ~11-13% for all the four impact categories. Among the several materials that compose the infrastructure, *Stainless steel* is the one that brings the largest impact for *Infrastructure* step, in the order of ~50-65%.

Cleaning and Sanitization step instead has an impact of ~ 8% GWP100, ~21% in Water use, ~8% in Acidification and, ~15% in Eutrophication. The impact derives primarily from the Citric acid, the wastewater disposal and from the electricity consumption.

Centrifugation phase has moderate impact. The shares are ~6% for GWP100, ~8% for Water use, 3% for Acidification and, ~ 35 % for Eutrophication. The impact of centrifugation is principally related to the energy consumption and from the disposal of wastewater. The disposal of wastewater is particularly relevant in the Eutrophication impact category.

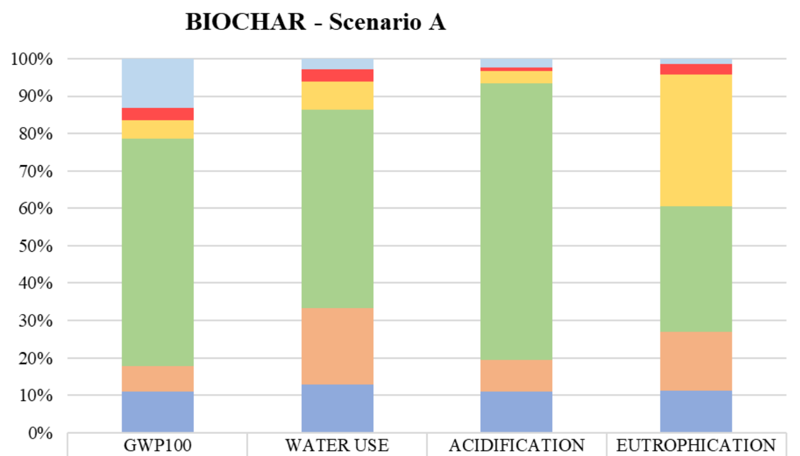
Following, the most impactful processes are *Bead milling* process, and respectively *Distillation* for Lipids and *Hydrothermal Carbonization* for Biochar production. *Bead milling* shows share of impacts of ~3- 4% (GWP100 and Water use), ~2.5% (Eutrophication) and ~1% (Acidification), mainly driven by the electricity consumption. *Distillation* phase presents an impact of ~4% in GWP100 and ~1-2% for Water use, Acidification and Eutrophication. Instead, HTC step presents a higher impact in the GWP100, ~13%, while for the other impact categories it is ~ 2%. In both processes impacts are driven by the energy consumption.

Instead, *Wet hexane extraction* has a negligible impact on the whole process, with a share of ~0% in each impact category. This is because this step involves no energy consumption and only a minor amount of hexane use and loss (as mentioned in *Chapter 3*).



	GWP100	WATER USE	ACIDIFICATION	EUTROPHICATION
Distillation	3,83%	1,75%	0,80%	1,23%
wet hexane extraction	0,0241%	0,0424%	0,0203%	0,0105%
bead milling	3,52%	3,44%	1,10%	2,64%
Centrifugation	5,53%	7,45%	3,17%	35,43%
Cultivation	67,48%	53,73%	75,24%	33,72%
Cleaning and sterilization	7,46%	20,51%	8,41%	15,73%
Infrastructure	12,16%	13,08%	11,26%	11,23%

Figure 18 Scenario A- Lipids, LCIA results with relative share of each process



	GWP100	WATER USE	ACIDIFICATION	EUTROPHICATION
HTC	13,20%	2,75%	2,27%	1,49%
Wet hexane extraction	0,0218%	0,0420%	0,0200%	0,0104%
Bead milling	3,18%	3,40%	1,08%	2,64%
Centrifugation	4,99%	7,38%	3,12%	35,34%
Cultivation	60,90%	53,19%	74,13%	33,63%
Cleaning and sterilization	6,73%	20,30%	8,29%	15,69%
Infrastructure	10,97%	12,94%	11,09%	11,21%

Figure 19 Scenario A- Biochar, LCIA results with relative share of each process

Focusing on the cultivation GWP100 impact alone, the major contribution (~46 %) derives from the transport of wastewater from the wastewater treatment plant to the microalgae production plant. Following, fertilizers have a total impact of ~33%, where 32% derives from the sodium nitrate and 1% from inorganic phosphorous fertilizer. Only ~21% of impact derives from energy consumption instead. These three fluxes, transport, fertilizers and electricity, are the ones that present the greatest impact in the cultivation phase also in the other impact categories, even if with different shares [Figure 20].

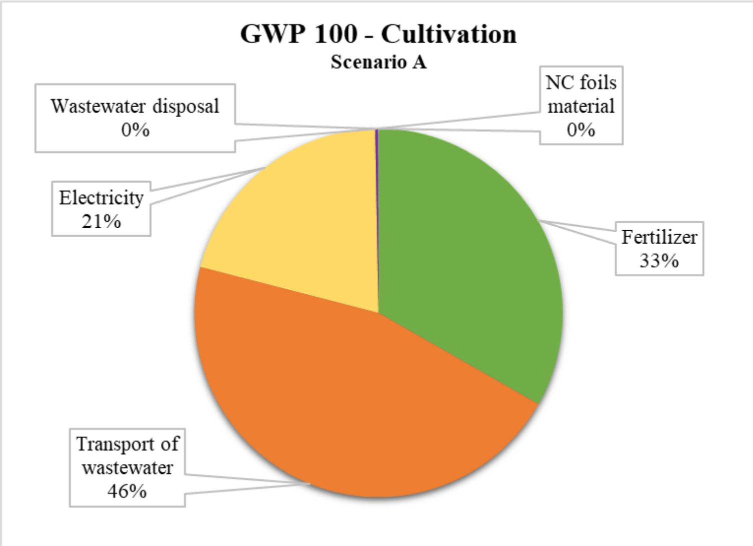


Figure 20 Scenario A - GWP100 quotas of cultivation flows

5.4.2 SCENARIO B

The second scenario studied, as mentioned in C*hapter 3,* exploited DAC technology to provide CO_2 and nutrients were supplied entirely through fertilizers.

Table 13 Scenario B - LCIA resultsshows the results for Scenario B under two frameworks: a complete framework and one where impact of *Infrastructure* and *Cleaning and Sanitization* steps are neglected.

Table 13 Scenario B - LCIA results

Scenario	Product	GWP100	WATER USE	ACIDIF.	EUTROP.
		$gCO_{2\ Eq}$/MJ	m^3/MJ	mol H^+_{Eq}/MJ	kg P_{Eq}/MJ
Scenario B	LIPIDS	291	0,0023	0,00110	0,0001
	BIOCHAR	425	0,0029	0,00144	0,0002
Scenario B w/o impacts of "infrastructure" and "cleaning and sanitization"	LIPIDS	213	0,0015	0,00069	0,00009
	BIOCHAR	326	0,0019	0,00092	0,00011

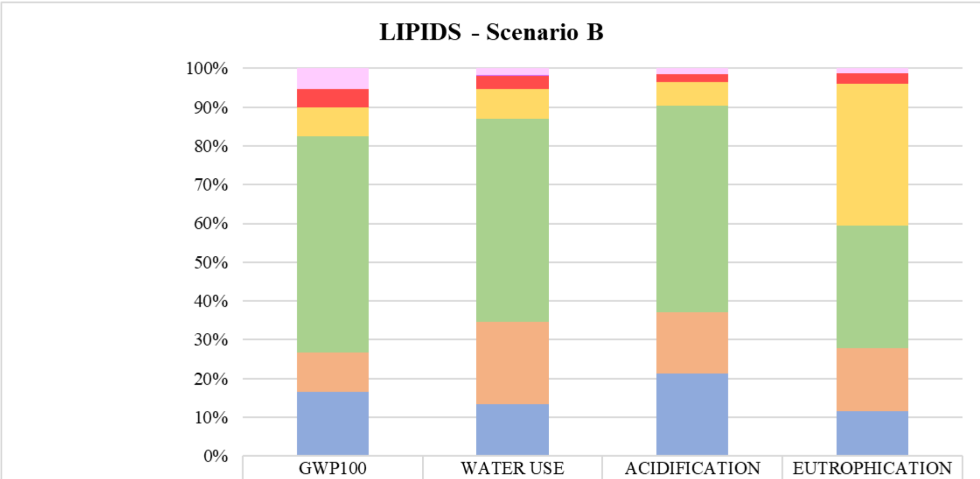
Figure 21 Scenario B- Lipids, LCIA results with relative share of each processand Figure 22 show the contribution of each process to the analyzed impact categories. From results, as in Scenario B, *Cultivation* phase has as significant impact. The share of its impacts are ~56% (GWP100), ~52% (Water use), ~53% (Acidification) and ~32% (Eutrophication).

Infrastructure has an impact of ~17% (GWP100), ~21% (Acidification), ~13% (Water use), and ~12% (Eutrophication). Among the several materials that compose the infrastructure, as mentioned above in Scenario A, *Stainless steel* is the one that brings the largest impact for *Infrastructure* step, in the order of ~50-65%. On the other hand, *Cleaning and Sanitization* step has an impact of ~10% in GWP100, ~21% in Water use, ~16% in Acidification and ~16% Eutrophication. The impact of *Cleaning and Sanitization step* derives primarily from the Citric acid, the wastewater disposal and the electricity consumption.

Subsequently, *Centrifugation* presents impacts of ~6-8% in GWP100, Water use, Acidification and, ~37% in Eutrophication. Impacts are primarily driven by electricity consumption and wastewater disposal. The latter is particularly relevant for the Eutrophication impact category.

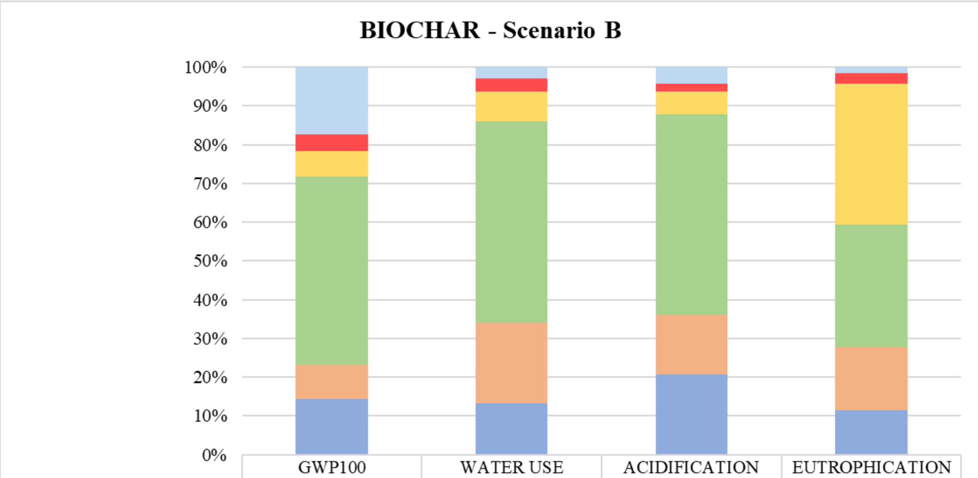
Following, there are *Bead milling*, *Distillation* for Lipids and *HTC* for Biochar. Bead milling shows shares of impact of about ~3.5-5% in GWP100 and Water use, while ~2-3% in Acidification and Eutrophication. *Bead milling* impact is mainly driven by the electricity consumption.

Distillation and *HTC* show a share of ~5% and ~17% respectively in GWP100 impact category, while in the other impact categories, *Distillation* presents an average share of ~1.5%, while HTC shows an average of 2-4%. Instead, *Wet hexane extraction* still shows an impact negligible in the process.



	GWP100	WATER USE	ACIDIFICATION	EUTROPHICATION
Distillation	5,22%	1,80%	1,52%	1,27%
wet hexane extraction	0,0328%	0,0436%	0,0383%	0,0108%
bead milling	4,79%	3,53%	2,07%	2,72%
Centrifugation	7,53%	7,66%	5,97%	36,51%
Cultivation	55,71%	52,45%	53,31%	31,70%
Cleaning and sterilization	10,16%	21,08%	15,87%	16,21%
Infrastructure	16,56%	13,44%	21,23%	11,58%

Figure 21 Scenario B- Lipids, LCIA results with relative share of each process



	GWP100	WATER USE	ACIDIFICATION	EUTROPHICATION
HTC	17,37%	2,82%	4,22%	1,54%
Wet hexane extraction	0,0286%	0,0432%	0,0372%	0,0108%
Bead milling	4,18%	3,50%	2,02%	2,72%
Centrifugation	6,57%	7,58%	5,81%	36,41%
Cultivation	48,56%	51,91%	51,84%	31,61%
Cleaning and sterilization	8,86%	20,86%	15,43%	16,16%
Infrastructure	14,44%	13,30%	20,65%	11,55%

Figure 22 Scenario B- Biochar, LCIA results with relative share of each process

Focusing on GWP100 alone in the *cultivation* phase, the major contribution, ~58 %, derives from the exploitation of fertilizers, 55% form sodium nitrate and 3% from inorganic phosphorous fertilizer. Following, energy consumption has an impact of ~34 %. Fertilizers and electricity are the ones that present the most relevant impact in the cultivation phase also in the other impact categories, even if with different shares. However, in Eutrophication impact category, tap water and disposal of wastewater gain relevance, respectively ~12% and ~8% [Figure 23].

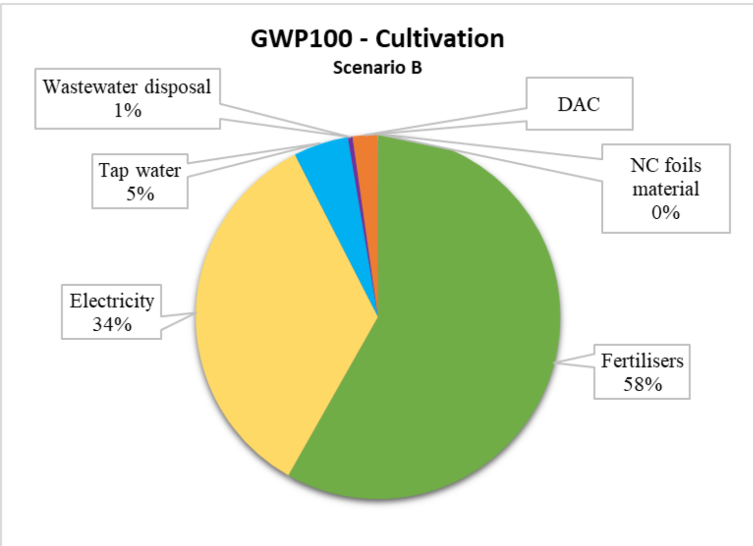


Figure 23 Scenario B - GWP100 quotas of cultivation flows

5.4.5 Comments and discussion

Table 12 Scenario A - LCIA results and Table 13 report results of the analysis with respect to the FU (1 MJ of lipids).

Concerning the GWP100, the environmental hotspot in Scenario A is mainly in the *Cultivation* phase. The C*ultivation* phase accounts for approximately 340 g CO_2-eq/MJ, with the main contribution, ~46%, arising from the transportation of wastewater from the wastewater treatment plant to the microalgae production facility. The wastewater used in this process is municipal; however, this impact could be reduced by utilizing wastewater from dairy or food-processing industries, as it typically contains higher nutrient concentrations, to reduce the amount of transported wastewater while guaranteeing the same nutrients supply. However, further investigation into the possible use of dairy and food industry wastewater for algae application is needed. Another possibility to potentially reduce the impact of wastewater transport could be the installation of the facility near the WW treatment plant or other plants producing WW suitable for direct use in the PBR. Following, fertilizers and electricity consumption show impacts of ~33% and ~21% respectively.

In scenario B, Cultivation phase still has a significant impact, ~56%, and so accounts for 162 g CO_2-eq/MJ. In this case, the largest impact in cultivation phase is driven by fertilizers, ~58%, and by electricity consumption ~34%.

In both scenarios, among the two fertilizers, sodium nitrate is the one that most significantly affects the impact categories. Therefore, improvements could be achieved through a more sustainable selection and supply of nutrients. As previously discussed, wastewater-derived nutrients represent a promising alternative; however, the type of wastewater and the mode of transportation can strongly influence the overall results. However, greater potential can be obtained both from optimizing transportation and fertilizer sources.

Instead, there is no relevant difference in the choice between DAC technology and Point source technology. As mentioned, environmental hotspots derive principally from electricity consumption and fertilizers.

So, in order to make technology sustainable, and also commercially feasible, improvements have to be implemented with the aim of reducing energy consumption and exploitation of fertilizers, in particular in the cultivation phase.

6 CASE STUDY – TEA

In this chapter, a brief review of the techno-economic aspects of lipid production from microalgae is presented. Particular attention is given to recent advancements and future developments in cultivation technologies, with a focus on photobioreactors (PBRs) as promising systems for large-scale and sustainable lipid production. The aim of this chapter is to establish a framework for understanding the techno-economic assessment (TEA) of microalgal lipid production and to provide a preliminary estimation of production costs. These considerations serve as a basis for evaluating the economic feasibility of microalgal lipids, and for identifying key factors influencing process performance and cost reduction potential.

6.1 Advancements in PBRs and downstream technologies

Large-scale microalgae cultivation requires maintaining optimal conditions such as light, nutrients, temperature, and pH. There are different cultivation technologies, each one with its advantages and disadvantages. Open systems, typically outdoor ponds, relieve natural sunlight and are cost-effective but vulnerable to contamination and environmental fluctuations. Instead, closed systems, such as photobioreactors (PBRs), provide controlled conditions and protection from contaminants; on the other hand, they present higher capital and operating costs. Hybrid approaches can combine the benefits of both, for example by initiating growth in closed systems before transferring cultures to open ponds. The choice of system depends on cultivation objectives, production scale, and economic feasibility. With technological advancement, optimization of cultivation strategies and novel applications are expected to further drive the development of the microalgae-based industry.

Beyond the system, microalgae cultivation efficiency depends on optimizing system design and operational factors such as nutrients supply, light, temperature, and harvesting methods, all crucial to maximize biomass yield and sustainability [66].

Closed photobioreactors (PBRs) offer clear advantages for microalgae cultivation, with technological advancements continuously improving their scalability, reliability, and cost-effectiveness, making them progressively suitable for both research and commercial applications. In general, PBR designs differ in efficiency, scalability, maintenance, adaptability, and associated costs (CAPEX and OPEX), underscoring the need to select the most appropriate configuration according to specific cultivation objectives and constraints [66].

Flat-panel PBRs provide efficient light distribution and simple scalability by connecting multiple panels, but they can be subjected to fouling and temperature control issues, which limit their application at larger scales. Tubular PBRs, both horizontal and helical, enable uniform illumination thanks to their small tube diameters and are easily scalable, making them suitable for outdoor operation and for applications such as biofuels, food, and feed production. Overall, these two configurations represent the most common closed systems for microalgae cultivation, each with specific trade-offs between cost, maintenance, and productivity [66].

From a TEA perspective, PBR choice directly influences both CAPEX and OPEX. Tubular PBRs present moderate CAPEX and moderate-to-high OPEX, which are mainly affected by the energy consumption. While Flat-panel PBRs feature simple maintenance requirements and high scalability potential connecting multiple panels. CAPEX are moderate-to-high and OPEX are moderate [66].

Thus, microalgae offer great potential for biofuels, food supplements, cosmetics, and environmental applications, but their commercialization remains limited by high production costs driven by energy demand, nutrient inputs, and infrastructure. Photobioreactor (PBR) innovations are crucial to improve efficiency, however, also downstream processing, harvesting and extraction, remain energy-intensive and costly, so improvements are required. Scaling up from lab to industrial scale, meaning an advancement in TRL level, can introduce non-uniform mixing, light limitation, temperature instability, and contamination risk. Among closed systems, flat-panel PBRs are easier to scale than tubular ones, while climate control remains essential for consistent productivity. Beyond technical issues, regulatory uncertainty, limited standardization, and consumer acceptance further restrict market growth. Establishing common operational standards and modular designs could enhance reproducibility and economic feasibility. Despite these barriers, advances in automation, renewable integration, and strain selection may soon enable cost-effective large-scale cultivation [66].

The integration of engineering, policy, and market development is key to unlocking the full potential of microalgae-based technologies.

Table 14 report the current development status of algal-based technologies [24].

Table 14 TRLs of different technologies involved in the production of lipids

Process	**TRL**
Cultivation	6-8 open pond 4-5 closed systems
Harvesting	6-9
Cell disruption	7-9
Extraction	7-9

6.2 Cost estimation

Concerning the current production costs for a flat-plate panel, *Tredici et al.* [67] reported detailed techno-economic assessment of a 1 ha Green Wall Panel plant located in Tuscany (Italy), assessing also the effect of scaling up to 100 ha.

The plant operated for 240 days/year under Mediterranean climatic conditions, with an average annual temperature of 16 °C. This resulted in an average annual productivity of 15 g m^{-2} d^{-1}, producing 36 t/year of dry biomass per hectare.

The Total Direct Capital Cost (TDC) was estimated to be 1,345,497 €, including GWP-II photobioreactor (37.5%), piping, fittings, valves, and tanks (~10.5%), machinery and equipment (28%), electrical equipment, instrumentation and controls (20%) and filed laboratory (4%). Total Indirect Capital Costs account for 216,280 €, considering engineering & supervision (5% of TDC), installation (10% of TDC) and taxes & insurance (1% of TDC + Land). Consequently, Fixed Capital Investments (FCI) is equal to 1,561,777 € and thus, resulting in a Total Capital Investment (TCI) of 1,661,777 €. Instead, the annualized capital charge was 101,260 €/year.

The operating costs (OPEX) comprise labor, fertilizers, electricity, consumables, maintenance, overhead and administrative expenses. In *Tredici et al.* [67] labor costs were taken from the average labor cost in Tuscany (Italy), considering a plant supervisor, a biologist and four workers (unskilled). Cost of fertilizers were assumed at 400 €/t for Sodium nitrate and 1500 €/t sodium dihydrogen phosphate, with respective annual consumptions of 15.3 tonnes/year and 1 tonnes/year respectively. Industrial electricity was priced at 0.175 €/kWh (including taxes and levies). Considering an annual energy consumption of 214,435 kWh/year, the electricity cost was 37,526 €.

Adding consumables, maintenance, overhead and administration costs, the total OPEX amounted to 345,107 €/year.

Considering both OPEX and the annualized capital charge, the biomass production cost was 12.4 €/kg_{DW}, referring to the process boundary from cultivation up to the wet paste at 20 % solids (centrifugation included).

Generally, the production cost of microalgae in commercial closed PBRs present an average of ~50 €/kg_{DW}, although this value strongly depends on the productivity of cultivated strain and operating condition.

Reported commercial costs vary widely, from about 5 $/kg to over 1000 $/kg, depending on system type and scale. The lowest costs are reported for *Dunaliella salina* in large natural ponds (Australia) and *Arthrospira* in open raceways (India, China).

For the flat-panel reactor, *Tredici et al* [67], as mentioned above, reported a biomass cost of 12.4 €/kg_{DW} achieved under sub-optimal climatic conditions (Tuscany). Even if this value is relatively low for a closed system, it is still too high for commodity applications such as biofuels.

Significant cost reduction can be achieved scaling up the plant to 100 ha due to strong economy-of-scale effects. CAPEX per hectares decreases by ~30%, following the scaling law (3) and applying a 0.85 scaling factor [67].

$$Cost\ B = Cost\ A * \left(\frac{Size\ B}{Size\ A}\right)^{0.85} \qquad (3)$$

At large scale, specific electricity costs drop from 0.175 €/kWh to 0.145 €/kWh, and fertilizer and consumable costs are reduced by approximately 30% per hectare. Similarly, also labor intensity reduces significantly.

As a result, the cost of biomass production in Tuscany decreases from ~12.4 €/kg at 1 ha to 5.1 €/kg at 100 ha, and further to ~4.1 €/kg when a thermo-tolerant strain is exploited to eliminate cooling needs. The cost structure also changes significantly with scale: at 1 ha, labor dominates (~40% of total costs), while at 100 ha, capital becomes the main contributor (~39%), followed by maintenance (~25%), electricity (~18%), and labor (~8%). Therefore, at industrial scale, economic performance is mainly determined by the ability to minimize capital expenditure through design simplification, material optimization, and modularity.

Results are summarized in Table 15.

Table 15 Comparison of costs from 1 ha and 100 ha Flat-Panel cultivation facility

Parameter	**Unit**	**Flat-Panel PBR**	
Scale	ha	1	100
CAPEX	M€/ha	1.6 M€/ha	0.8 - 1.0 M€/ha
OPEX	€/t AFDW	9 600 €/t AFDW	3 100 €/t AFDW
Cost of biomass	€/kg DW	12.4 €/kg DW	~5.1 €/kg DW ~4.1 €/kg DW (using a thermos tolerant strain)

Overall, scaling from 1 to 100 ha more than halves the production cost of microalgal biomass, shifting the focus from labor efficiency to capital optimization. In favorable climates (i.e, Tunisia), where energy and labor are cheaper and cultivation can occur all the year, total costs could reduce even further to 2.6 - 3.2 €/kg, confirming that large-scale deployment combined with suitable location and thermo-tolerant strains is the key to economic feasibility.

Nevertheless, according to *Tredici et al* [67] algal biomass cost lower than 1 €/kg will not be achieved in the near future without breakthroughs in strain characteristics (biological productivity) and cultivation technologies.

These cost levels represent the current achievable performance of flat-plate PBR systems. The following section reports target values derived from "*Techno-Economic Analysis for the Production of Algal Biomass via Closed Photobioreactors: Future Cost Potential Evaluated Across a Range of Cultivation System Designs*" [68], illustrating the potential cost reductions expected for fully optimized, industrial-scale ("nth-plant") systems.

The target scenario developed by NREL [68] is based on a set of techno-economic assumptions representing a fully industrialized and high optimized "nth-plant" facility. The analysis considers commercial scale photobioreactor plants with a total cultivation area between 100 and 500 hectares, operating year-round under favorable climatic conditions similar to those of the U.S. Gulf Coast, which offers high solar irradiance and mild temperatures.

The assumed areal productivity is 45–55 g m^{-2} d^{-1}, achievable through advanced strain selection, optimized light path, and efficient mixing. The biomass concentration prior to harvesting is fixed at approximately 2 g L^{-1}, allowing effective centrifugation with limited energy input.

Electricity is supplied at an industrial rate of 0.07 - 0.08 $/kWh (~0.06 - 0.07 €/kWh), and both CO_2 and nutrient sources (mainly sodium nitrate and phosphate) are assumed to be purchased in bulk at large-scale prices. The model assumes a high degree of process automation, resulting in low labor demand, while capital costs benefit from a mature supply chain and modular fabrication of PBR components (no first-of-a-kind penalties).

Financially, the analysis adopts a 20-year plant lifetime and a 10% internal rate of return (IRR). These assumptions collectively describe an optimized future industrial configuration, representing the lower bound of achievable production costs for closed photobioreactor systems.

All values are harmonized to €2024, and only tubular and flat-plate PBRs are considered here for comparison. Results are summarized in Table 16.

Table 16 Target cost in a fully industrialized and high optimized scenario

Parameter	Unit	Tubular PBR	Flat-plate PBR.
Areal productivity	g m^{-2} d^{-1}	45-55	45-55
Biomass concentration before harvesting	g/L	~2.0	~2.0
CAPEX	M€/ha	0.10 – 0.85 M€/ha (~0.11 – 0.93 M$2014/ha)	1.15 M€/ha (~1.26 M$2014/ha)
OPEX	€/t AFDW	350 – 540 €/t AFDW (~377 – 578 $2014/t)	450 €/t AFDW (~ 478 $2014/t)
Cost of biomass	€/kg DW	0,5 – 1,6 €/kg DW (= 0,64 – 1,79 $2014/kg)	0,6 – 1,6 €/kg DW

The NREL [68] target values summarize the techno-economic potential of closed PBRs under mature, industrial conditions. It highlights that most of the cost reduction comes from higher areal productivities, lower specific CAPEX through standardized fabrication, and reduced labor intensity via automation. However, these targets refer to an "nth-plant" scenario and should be interpreted as lower bound costs once the technology is fully optimized.

The next section will quantify the contribution of downstream operations, cell disruption and solvent extraction, to the lipid cost and investigates how lipid fraction and biomass productivity influence the final costs.

A fully industrialized and high optimized facility is assumed as starting point for the cultivation phase, in order to consider an industrial plant

The NREL target flat-plate PBR facility [68], is taken as reference. Energy consumption in bead milling, wet hexane extraction and distillation are the ones reported in *Chapter 5*. For coherence with LCA boundaries and considerations, only CAPEX of PBR infrastructure is considered (even if in this case also centrifuge is included), while in the other steps are considered only OPEX.

To evaluate cost of lipid production, including also downstream operation, Equation (4) is applied:

$$C_{lip} = \frac{CAPEX*CRF+ OPEX_{ann}}{f_{lip}*\eta_{extr}} = \frac{MBSP_{DW}+OPEX_{bead\ milling+hexane\ extr}}{f_{lip}*\eta_{extr}} + OPEX_{distill.} \quad (4)$$

Where:

- CAPEX: capital expenditure of PBR;

- CRF: Capital Recovery Factor = $\frac{i(1+i)^n}{(1+i)^n-1}$ with *i* tax rate and *n* life of the plant;
- $OPEX_{ann}$: annual operative expenditure;
- $MBSP_{DW}$ Minimun Biomass Selling Price.

For this preliminary cost estimation, the following assumptions were applied.

The Flat-panel target scenario [68], representing a fully industrialized and high optimized "nth-plant" facility, presented a MBSP od Dry Weight biomass of ~0.6 – 1.6 €/kg_{DW}. The lipid content of the microalgal strain was assumed to be 25 % of dry weight, and the hexane extraction efficiency was fixed at 95 %, in accordance with the assumptions reported in *Chapter 5*. The contribution of hexane to operating costs was considered negligible. Based on the measured solvent loss of approximately 2.4 g of hexane per kg of dry biomass, and assuming a market price of 1.5–2 €/kg, the resulting cost impact is below 0.02 €/kg of lipids.

Electricity and thermal consumption for downstream processes are assumed to be equal to the ones reported in *Chapter 5* and reported in Table 17.

The industrial electricity price was taken as 0.2399 €/kWh [69], including all taxes and levies, corresponding to the average Dutch industrial tariff, to maintain coherence with geographical boundaries of the preliminary LCA (*Chapter 5*). Instead, thermal energy for solvent recovery (distillation) was assumed to be provided by natural gas at an industrial rate of 0.0757 €/kWh [70], including all taxes and levies, which corresponds to the average price for non-household consumers in Dutch, also in this case to maintain coherence with geographical boundaries adopted in LCA preliminary analysis.

Another remark should be made concerning the cost allocation approach. As discussed in *Chapters 3 and 5*, in addition to lipids, the process also can yields co-product, such as high-value product and biochar, derived from the residual biomass.

Therefore, following the energy-based allocation criterion (44 % to lipids and 56 % to biochar Table 6) the lipid cost can be expressed as (5):

$$C_{lipids} = \frac{MBSP_{DW} + OPEX_{bead\ milling + hexane\ extr}}{f_{lip} * \eta_{extr}} * \alpha_{lipids} + OPEX_{distillation} \qquad (5)$$

where f_{lip} is the lipid mass fraction (25 %), η_{extr} is the extraction efficiency (95 %), and $\alpha_{lipids}$is the allocation factor. All results are shown in Table 17.

However, allocation can be performed also on other basis (mass basis, economic basis, and others).

Table 17 Lipid production cost in a fully industrialized and high optimized scenario

Process	**Flat-Panel PBR** **Target scenario – fully industrialized and high optimized**	
PBR and centrifugation	0.6 – 1.6 €/kg_{DW}	2.53-6.73 €/kg_{lipids}
Bead milling – electricity	0.694 kWh/kg_{DW}	0.511 €/kg_{lipids}
Distillation – electricity	0.54 kWh/kg_{DW}	0.095 €/kg_{lipids}
Distillation – Natural gas	1.75 kWh/kg_{DW}	0.109 €/kg_{lipids}
Cost of lipids with energy allocation		1.54 – 3.39 €/kg_{lipids}

All results are presented referring to kg of lipids. In the preliminary LCA the functional unit was 1 MJ of lipids. The conversion from lipid-mass specific costs to lipid-energy specific costs can be easily performed considering the LVH of lipids as 38.3 MJ/kg [19]. However, it is important to notice that fluxes, both in LCA and TEA are significantly dependent on the lipid content; in this case for sake of simplicity, costs are considered on kg of lipids.

Thus, from this preliminary cost estimation, the cost of lipids production results in 1.54 – 3.39 €/kg_{lipids}.

In Table 18 is reported a sensitivity analysis of the lipid production cost with respect to the lipid mass fraction. To isolate the compositional effect, the energy-based allocation factor was kept constant at 44%, even if actually, the change in composition also affects the energy allocation factor. From this sensitivity it can be observed that, isolating the allocation factor and keeping it constant, at the increasing of the lipid content, the production costs of lipids decrease.

Table 18 *Sensitivity analysis of cost of lipids varying lipid fraction*

f_{lipids} [kg_{lipids}/kg_{DW}]	**Cost** [€/ kg_{lipids}]
0.20	1.89 – 4.21
0.25	1.53 – 3.39
0.30	1.30 – 2.84
0.35	1.13 – 2.45
0.40	1.00 – 2.16

Nevertheless, the aim of this work was to establish an average proximate value for production costs of lipids starting from literature, which resulted to be 1.54 – 3.39 €/kg_{lipids}.

Instead, concerning the SUN-PERFORM project, this TEA provides a reference, estimating a preliminary cost of lipids. The possible valorization of the residual part of the biomass was not considered. Thus, potential benefits from high-value products and from production of biochar and its potential exploitation as soil amendment, were not included. However, further research and assumption will be established to perfume a more comprehensive TEA analysis.

7 Conclusions

Microalgae represent a potential resource for the development of sustainable bioproducts. They can be cultivated on non-arable land, contribute to CO_2 capture and exhibit a high lipid content, making them particularly suitable for the production of advanced biofuels. The lipids extracted from microalgae can be directly converted into fuels, possibly eligible as Sustainable Aviation Fuels (SAFs), thereby contributing to the de-fossilization of the aviation sector.

This work provided an overview of the state of the art of technologies for lipid production from microalgae, examining the main cultivation, harvesting and extraction systems. Subsequently, a preliminary Life Cycle Assessment (LCA) and a Techno-Economic Analysis (TEA) were carried out.

While the LCA methodology benefits from existing guidelines, the TEA requires a case specific tailoring. In both methodologies, if not guided by specific standards, several key decisions still rely on the practitioner, such as for the functional unit definition, the system boundaries and the allocation. The goal of integrating the two approaches is to identify a trade-off between environmental and economic sustainability, supporting the development of technologies that are feasible on both fronts.

However, integrating LCA and TEA still presents several challenges; as mentioned, there can be inconsistencies in the choice of system boundaries and functional units between the two methodologies. Current research efforts are therefore directed toward developing integrated assessment tools that can bridge existing gaps.

This study was developed in the framework of the EU supported SUN-PERFORM project. The project aims to achieve a breakthrough in the production of lipids from microalgae by implementing biologically modified algal strains and NC foils designed to enhance light capture, increase photosynthetic efficiency and, consequently, increase productivity. Moreover, the implementation of NC foils will reduce IR irradiation of the reactor, thus reducing the overheating of the reactor and therefore reducing the energy consumption tied to reactor cooling. Besides the increase of productivity, the genetic modification of the algae strain is expected to increase the lipid fraction. Thus, energy consumption required to produce 1 MJ of lipids, which is one of the critical factors, will decrease, and consequently the costs.

As a first step, a preliminary LCA was performed considering two distinct scenarios:

- Scenario A: in which CO_2 is from a point-source and nutrients supplied by wastewater and fertilizers;
- Scenario B: CO_2 is obtained by Direct Air Capture (DAC) technology and nutrients are supplied with fertilizers.

Initially, a tubular photobioreactor (PBR) was selected as cultivation technology. However, literature data indicate that tubular PBRs are highly energy-intensive (up to 29 kWh/MJ), making them unsuitable for large-scale production. In the framework of the SUN-PERFORM project, PBR are optimized by means of innovative technologies such as tailored solar foils. However, due to the lack of available data, a flat-plate PBR was chosen as a better proxy for the analysis. The LCA for lipid production resulted in a global warming potential (GWP100) of approximately ~396 gCO_2eq/MJ_{lipids} for Scenario A and ~291 gCO_2eq/ MJ_{lipids} for Scenario B.

In Scenario A, a significant portion of the impact (~38%) is associated with the transport of wastewater, which could be potentially mitigated by selecting a wastewater which is richer in nutrients sources and adopting more efficient transport modes. Alternatively, it can be reduced by locating the facility near a wastewater treatment plant or other plants producing WW suitable for direct use in the PBR. In both scenarios, however, fertilizer use, and electricity consumption remained the main environmental hotspots.

The obtained results are not yet competitive with fossil-based fuels (~89 g CO_2 eq/MJ according to CORSIA). Nevertheless, this study tried to identify the key improvement areas required to enhance the sustainability of microalgae-lipid production. These improvements include reducing energy consumption in the cultivation phase (e.g., by adding solar foils to the PBRs, etc.), employing more sustainable nutrient sources and increasing system productivity also through an implementation of biological improvements in the algal strains.

Applying these strategies is expected to significantly lower the overall environmental impact, moving PBR closer to a technologically feasible and environmentally sustainable production of algal lipids for future biofuel applications.

From the TEA perspective, several factors influence the final production cost of lipids from microalgae. Among the most relevant, there are biomass productivity, lipid content and technology employed. Improving these aspects, for example, adopting different fertilizers or implementing biological improvements in the algae strain, can lead to a reduction in lipid production cost. This approach is in line with the expectation of the SUN-PERFOM project. Another significant aspect is the location selected for the production: some of the OPEX are highly dependent on the location, such as labor cost and cost of electricity. Based on a preliminary TEA, that considers a fully industrialized and high optimized facility, the estimated production cost for the lipid fractions resulted in ~1.5-3.4 €/kg_{lipids}. However, this preliminary value did not consider the possibility to valorize the residual part of the biomass, targeting high-value products and potentially the production of biochar and its potential valorization as soil amendment. In future, through a more comprehensive development of this analysis, the role of highly valuable product will be integrated, and their potential environmental and economic benefits will be assessed.

In conclusion, this study represents a starting point for further developments within the SUN-PERFORM project. The adoption of less energy-consuming PBR configuration, the exploitation of more sustainable fertilizer and an increase in productivity are potential solutions that can reduce the environmental impacts and the cost of lipid production from microalgae.

As planned, the advancements that will be developed in the SUN-PERFOM project are expected to significantly improve both the environmental and economic performance of microalgae-based lipid production, leading towards more sustainable and competitive biofuel technologies.

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