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Cascading *Crypthecodinium cohnii* Biorefinery: Global Warming Potential and Techno-Economic Assessment

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Abstract: Prior to the commissioning of a new industrial biorefinery it is deemed necessary to evaluate if the new project will be beneficial or detrimental to climate change, one of the main drivers for the sustainable development goals (SDG) of the United Nations. In particular, how SDG 7, Clean and Efficient Energy, SDG 3, Good Health and Well Being, SDG 9, Industry Innovation and Infrastructure, and SDG 12, Responsible Production and Consumption, would engage in a new biorefinery design, beneficial to climate change, i.e., fostering SDG 13, Climate Action. This study uses life cycle assessment methodology (LCA) to delve in detail into the Global Warming Impact category, project scenario GHG savings, using a conventional and a dynamic emission flux approach until 2060 (30-year lifetime). Water, heat and electricity circularity are in place by using a water recirculation process and a combined heat and power unit (CHP). A new historical approach to derive low and higher-end commodity prices (chemicals, electricity, heat, jet/maritime fuel, DHA, N-fertilizer) is used for the calculation of the economic indicators: Return of investment (ROI) and inflation-adjusted return (IAR), based upon the consumer price index (CPI). Main conclusions are: supercritical fluid extraction is the hotspot of energy consumption; *C. cohnii* bio-oil without DHA has higher sulfur concentration than crude oil based jet fuel requiring desulfurization, however the sulfur levels are compatible with maritime fuels; starting its operation in 2030, by 2100 an overall GHG savings of 73% (conventional LCA approach) or 85% (dynamic LCA approach) is projected; economic feasibility for oil productivity and content of 0.14 g/L/h and 27% (w/w) oil content, respectively (of which 31% is DHA), occurs for DHA-cost 100 times higher than reference fish oil based DHA; however future genetic engineering achieving 0.4 g/L/h and 70% (w/w) oil content (of which 31% is DHA), reduces the threshold to 20 times higher cost than reference fish oil based DHA; N-fertilizer, district heating and jet fuel may have similar values then their fossil counterparts.

Keywords: DHA nutritional supplement; advanced fuel; district heating; N-fertilizer; water and energy circularity; conventional and dynamic LCA; net zero carbon electricity 2050

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1. Introduction

Short-term and long-term carbon cycles are the key to exclude fossil fuels from human activities energy and chemicals production and opt for alternative sources of carbon: biomass waste or microalgae. There are four main reservoirs of carbon: atmosphere, ocean, land (plant and soil), and geological reservoirs (fossil fuels and rock carbonates) [1]. Humans, as well as other heterotrophic organisms, are carbon sinks (carbon stored in their structure) and sources (respiration). Human activities are based on a carbon economy for products and energy: products have carbon in their constitution, for example automotive parts, packaging and toys with polypropylene (C_3H_6)n; textiles with mainly cellulose ($C_6H_{10}O_5$)n (cotton, lyocell, viscose, rayon, SeaCell), and alanine $C_3H_7NO_2$ (silk) [2]; energy from combustion uses fuels with carbon (for example natural gas (mixture of CH_4, C_2H_6, CO_2) [3,4], wood pellets for heating or wood in furniture/houses (cellulose ($C_6H_{10}O_5$)n). At some point in its life cycle the use of the carbon is released into the atmosphere in the form of CO_2 at the end-of-life of the product/energy by combustion/incineration. Even in landfilling of products, both CO_2 and CH_4 are emitted due to bacterial decomposition with or without oxygen [5].

Without human interference, the carbon in fossil fuels would leak slowly into the atmosphere through volcanic activity over millions of years in the slow carbon cycle (long-term carbon cycle). Burning fossil fuels and clearing land has two coinciding effects that increase carbon in the atmosphere: the former releases carbon that was stored millions of years ago, and the latter removes forest, eliminating plants that would otherwise take carbon out of the atmosphere as they grow. Bioenergy systems are assumed to be carbon neutral on the basis that the change of carbon stock from lithosphere to atmosphere is more detrimental than from atmosphere to land. However, this assumption ignores the time lag between CO_2 emissions from biomass combustion and CO_2 uptake by vegetation [6]. Even if this affects climate change, temporarily, as the short-lived climate forcers ground level ozone and black carbon [7], it has a shorter effect than fossil combustion emissions [6].

Reaching net carbon removals is facilitated by bio-based systems (biorefinery) with CO_2 sequestration. If the target is net zero GHG emissions, higher amounts of CO_2 must be removed and it will take longer to achieve zero. For example, in 1.5 °C scenarios, net-zero CO_2 occurs almost two decades before net-zero GHG (≈2050 vs. 2067). In 2 °C scenarios, net-zero CO_2 occurs roughly three decades before net-zero GHG (≈2070 vs. 2100). Biorefinery systems with carbon storage in lithosphere or in long-lived materials will promote net carbon removals [8,9].

The transition from a fossil-based economy to a bio-based economy is necessary if an equilibrium is targeted avoiding displacing carbon from the long-term cycle to the short-term carbon cycle. Renewables in electricity generation (eolic, solar, hydro) rely on abiotic materials that are produced/transported via fossil fuels. Abiotic materials from biomass waste and without requiring fossil energy (bio-products) should also be preferable. The transition from a fossil-based economy to a bio-based economy should be aligned with the United Nations Sustainable Development Goals (UNSDG) [10] and underpin biorefinery projects relevance.

One of the drivers for the transition is the EU ETS (European Emission Trading Scheme), that sets benchmark stack fossil CO_{2eq} emissions for high fossil fuel emitters [11]. The EU ETS [12] began in 2005 and covers emissions from power, industry, and flights inside the EEA, around 40% of EU emissions. It is currently the world's largest domestic carbon market. Min value: 2.68 EUR/tonCO_{2eq}, max value: 64.32 EUR/tonCO_{2eq} and average value: 14.51 EUR/tonCO_{2eq} in 2008–2020, however in 2021 the historical maximum of 88.88 EUR/tonCO_{2eq} (see Figure 1) was reached.

The relationship between biomass, the energy transition, and the circular economy is by means of a biorefinery in a bioeconomy. Accelerating the transition to a bioeconomy in the context of sustainable development goals is deemed necessary.

The main issues regarding a biorefinery project's sustainability assessment rely on the lack of a worldwide harmonized methodology to assess its future advantage in comparison with conventional production methods, already established at industrial level and achieving similar products. In fact, the life cycle methodology guidelines (LCA-Life Cycle Assessment, ISO 14040 [13]) are very useful but have limitations: depending on the boundaries, allocation, and hypothesis used for each input and output of the biorefinery, different electricity mixes (year and location dependent), different CO_2 equivalency factors (updated by IPCC reports), and use of databases with old processes, lead to significant differences in the outcomes of the analysis and make comparisons between studies very hard. There is a need for a harmonized approach ensuring transparency, and credible and open accessibility to all the investors, engineers, scientists, and decision makers, and ensuring replicability by a third party. Besides the environmental sphere, the investment attractiveness should also rely on the same principles. The need to compare the bioenergy scenario with a reference 'no-bioenergy' scenario that delivers the same services to society is underpinned by Cowie et al., 2021 [14].

Figure 1. One of the drivers for the energy transition the CO_{2eq} tax (EUR CO_{2eq}/ton) volatility in 2021 (https://ember-climate.org/data/carbon-price-viewer/, accessed on 1 January 2022).

The marine heterotrophic microalgae *Chrypthecodinium cohnii* produces significant amounts of intracellular lipids that are rich in docosahexaenoic acid (DHA), a compound that has many applications in the pharmaceutical, nutraceutical, and food industries, due to its well-known benefits on human health. The *C. cohnii* oil fractionation, to obtain a DHA rich fraction, and another lipidic fraction, rich in saturated/unsaturated fatty acids, that can be directed towards biodiesel purposes, is a possible approach to valorize all the microalgae oil fractions, which may have environmental benefits. Because the biodiesel market is expected to slow down/decrease significantly, due to road transport electrification, in the future the needs of bio-maritime and bio-jet fuels will be the main target, and the main players in the biofuels market [15]. To ensure the biorefinery is flexible in terms of biofuel products, a catalytic HDO (hydrodeoxygenation) and HDS (hydrodesulfurization) unit are assumed to promote oxygen reduction in the form of water molecule, decarboxylation, hydrogenation, hydrogenolysis, and hydrocracking at mild conditions of temperature and pressure that in turn release oxygen in the form of CO and CO_2, respectively, causing a reduced number of carbon in the hydrocarbon product [16].

Considering these issues, this research focuses on deriving a methodology to guide the GHG emissions estimation and a techno-economic (TEA) assessment of a new biorefinery based on microalgae *C. cohnii*, targeting the microbial production of DHA, maritime/jet fuel, N-fertilizer, and district heating, with water, heat and electricity circularity. In addition, the concept of the *C. cohnii* biorefinery has undergone a preliminary screening regarding social aspects, to ensure the technology develops in a satisfactory way for the consumers and society at large. An Excel spreadsheet model for GHG, for TEA and Python code is provided in the Supplementary Materials.

2. Materials and Methods

The analysis focuses on a prospective *C. cohnii* biorefinery producing four products: DHA, maritime/jet fuel, N-fertilizer, and district heating. To devise the 2030–2050 *C. cohnii* prospective supply, a framework based on international organizations must be established. The framework in 2030–2050 will have different population growth, different nutraceuticals (omega-3) demand, and different maritime/aviation fuel needs in terms of advanced biofuels and sustainability criteria. Besides producing sustainable products, in comparison with the fossil-based counterpart, targeting climate action, the biorefinery itself should be aligned with the other UNSDG [17], i.e., promote circularity (responsible production) and foster industry, innovation, and infrastructure, such as depicted in Figure 2.

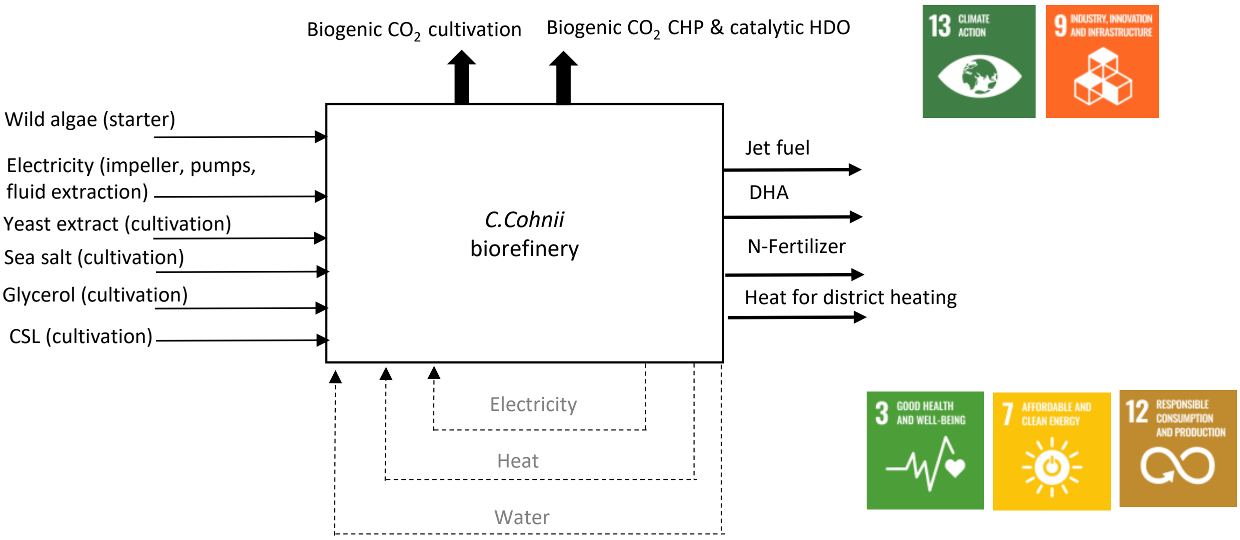


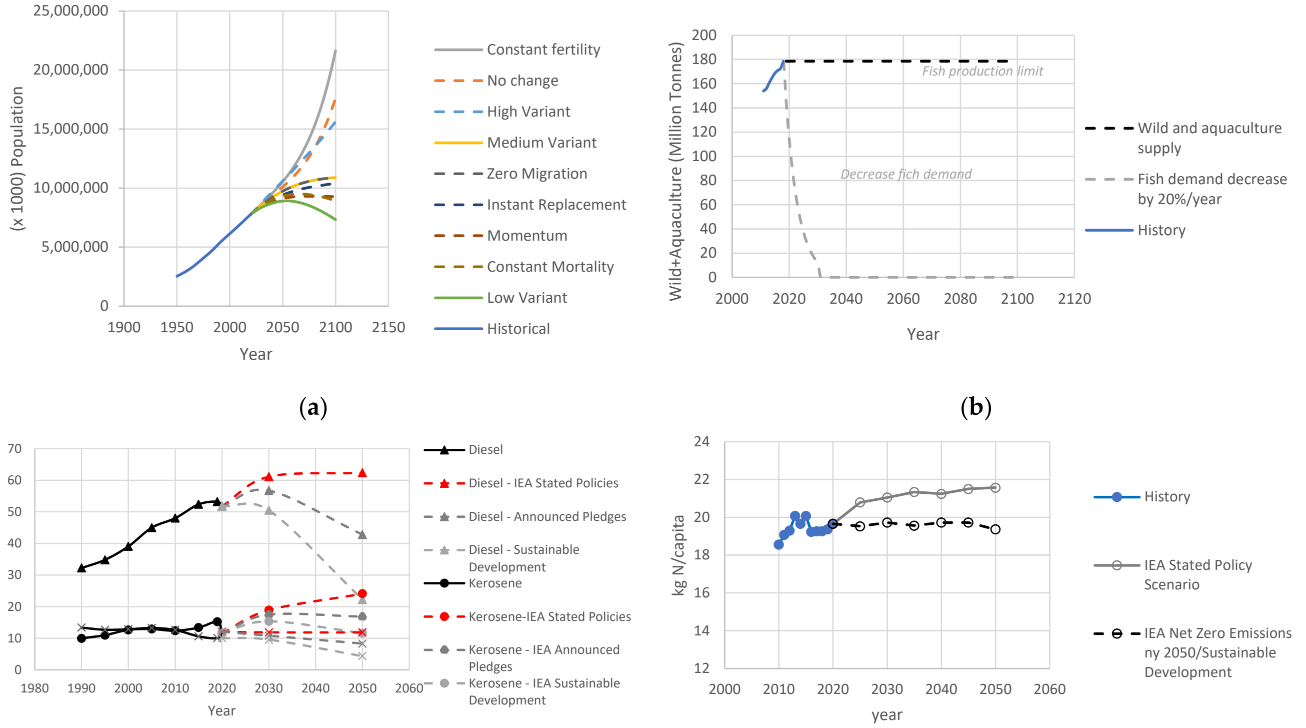
Figure 2. *C. cohnii* biorefinery and UNSDG relationship (own reasoning).

Regarding aviation and maritime needs for advanced and sustainable fuels, the ICAO (International Civil Aviation Organization, Montreal, QC, Canada)—CORSIA (Carbon Offsetting and Reduction Scheme for International Aviation) policy framework on aviation biofuels states that by 2027 all members must use up to 50% sustainable aviation fuels (SAF), which have to have environmental benefits in terms of a net CO_2 reduction of at least 10% compared to the baseline life cycle emissions values for aviation fuel, equal to 89 gCO_{2e}/MJ. Hydroprocessed esters and fatty acids (HEFA) allowed so far are tallow, global used cooking oil, global palm fatty acid distillate, global corn oil (from dry mill ethanol plant), USA soybean oil, Brazil soybean oil, EU rapeseed oil, Malaysia and Indonesia palm oil, Brazil *Brassica carinata* (grown as a secondary crop that avoids other crops displacement), and USA *Brassica carinata* (grown as a secondary crop that avoids other crops displacement), with 13.9 gCO_{2eq}/MJ for the cooked oil and 99.1 gCO_{2eq}/MJ for the palm oil (including indirect land use change). The standard regulating the technical certification of SAF is ASTM D7566. It can be blended up to the maximum certified blending limit. Upon release from blending, the fuel is certified to ASTM D1655 and from this point is regarded as conventional Jet A or Jet A1 kerosene. Hydroprocessed hydrocarbons from the algae (*Botryococcus braunii*) have a maximum allowed blend of 10% (% v/v).

The International Maritime Organization (IMO) targets a 70% reduction in the CO_2 intensity by 2050, maintaining the low sulfur needs. Maersk's 700+ container ships consume 10 million ton fuel oil/year and emit 0.1% of global CO_2 emissions and is currently the largest source of anthropogenic sulfur emissions. From 2020, the IMO have restricted the maximum allowed sulfur content of marine fuels used in international waters from 3.5 wt.% to 0.5 wt.% [18]. The advantage of marine fuels is that the requirements are of lower quality and do not need intensive upgrading and refining; nevertheless, either the current fossil standard ISO 8217-2017 will need to be modified to incorporate the novel fuel and its characteristics, or, a separate new standard could be developed, which could take several years. While microalgae has been shown to be a promising source of plant-based oils with high productivities, the technology to produce commercial scale algal oil at competitive prices is not yet available [18]. Mostly expected to be diesel engines with up to 20% blend. No sustainability criteria are defined yet.

One of the initiatives being assessed under the European Green Deal is the Renewable Energy Directive 2018/2001/EU (RED II) which is the main regulatory framework for EUs renewable energy. In the RED II (criteria recognized for EU ETS) framework, renewable energy supplied to the maritime and aviation sectors are eligible for counting towards the 14% renewable energy in transport target for road transport in 2030 (by a factor of 1.2). The RED II poses a limit on food and feed crops of 2020 level of 7%. There is also an upper limit of 1.7% for biofuels produced from Annex IX-B feedstocks (used cooking oil and animal fats) and a sub-target with minimum of 3.5% in 2030 for advanced biofuels produced from Annex IX-A feedstocks (waste materials and residues). The difference between CORSIA and RED II are pointed out in [19], for example, the input used for calculations and the absence of terms for emissions for carbon capture and storage and emissions from soil carbon accumulation via improved agricultural management.

Regarding DHA nutritional needs, most recommendations are for a daily intake in the range of 0.25–0.5 g per person per day [20]. Regarding N-fertilizer, it is expected to be used in a roughly constant value of around 20 kgN/capita in 2050, similar to 2010 values [21]. By 2050, the demand for nitrogen per capita increases by 10% in the Stated Policies Scenario. In the Sustainable Development Scenario and Net Zero Emissions by 2050 Scenario, the demand per capita is similar to today, despite total nitrogen use increasing by 25%. Figure 3 shows the historical and future framework for the biorefinery. The needs of the biorefinery products will be based on these constraints (Figure 4).



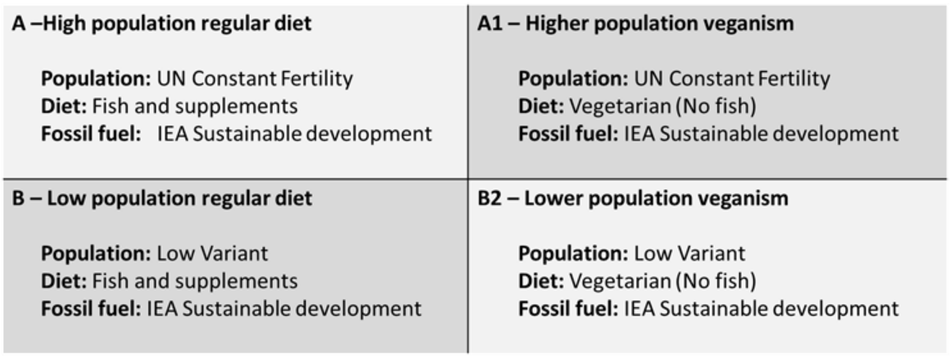
(**a**) (**b**)

(**c**) (**d**)

Figure 3. Constraints for future needs, own elaboration: (**a**) history and scenarios for population evolution [22], (**b**) history and scenario for fish oil consumption [23,24], (**c**) history and scenarios for fossil fuels in transportation [25], (**d**) history and scenarios for N-fertilizer [21].

2.1. Bench-Scale Inventory and Scale-Up Considerations

Wild strain *C. cohnii* ATCC 30772 was characterized, according to ISO 16948, and its ultimate analysis is (in wt.%), 41.2 C, 5.1 H, 4.7 N and 1.2 S. The starter vial, inoculum vial, and batch reactor have to be heated and agitated, consuming heat and electricity, carbon, and nutrients (Figure 5). The latter are used to take advantage of industrial by-products [26] and the medium is composed of industrial glycerol (supplied by IBEROL, a Portuguese biodiesel producer) as the carbon source (24.52 g/L), sea salt (25 g/L), yeast extract (2 g/L), and corn step liquor (supplied by COPAM, a national enterprise for starch production), as the nitrogen source, (4.59 g/L). The antibiotic is composed of (chloramphenicol (5 mg/L), penicillin G (62 mg/L), and streptomycin (100 mg/L)). Based on the ultimate analysis, it is expected that the biogenic CO_2 release be, according to our previous findings [27], 0.412 kg C/kg dry algae × 0.9 $kgCO_2$/kg C = 0.4 $kgCO_2$ biogenic/kg dry algae. After the inoculum preparation, a 7 L batch reactor, with Rushton impeller, is fed by medium and antibiotic. Antibiotic is needed at this stage but not in scale-up batch reactors.



A –High population regular diet	**A1 – Higher population veganism**
Population: UN Constant Fertility **Diet:** Fish and supplements **Fossil fuel:** IEA Sustainable development	**Population:** UN Constant Fertility **Diet:** Vegetarian (No fish) **Fossil fuel:** IEA Sustainable development
B – Low population regular diet	**B2 – Lower population veganism**
Population: Low Variant **Diet:** Fish and supplements **Fossil fuel:** IEA Sustainable development	**Population:** Low Variant **Diet:** Vegetarian (No fish) **Fossil fuel:** IEA Sustainable development

Figure 4. Analyzed frameworks for *C. cohnii* supply (higher-end needs).

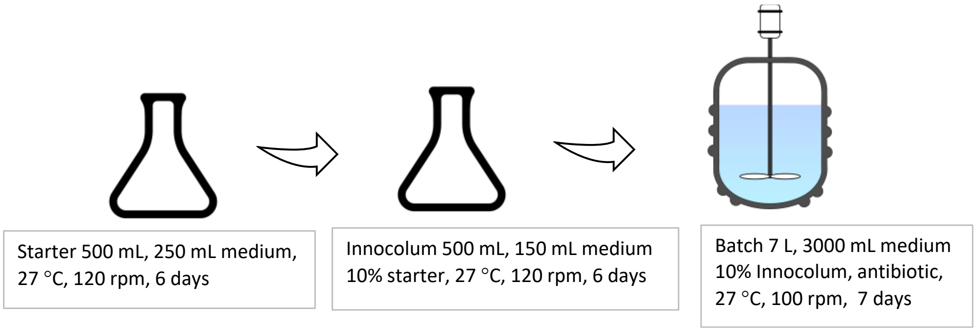


Figure 5. Cultivation step. Starter, inoculum, bench-scale batch reactor.

After 7 days, the culture is harvested and freeze dried (5% moisture). The energy consumption of the dryer is 2 $kWh/kg_{removed\ water}$ [28]. The dry algae are subjected to supercritical fluid extraction, consuming electricity and heat, to separate DHA ($C_{22}H_{32}O_2$) from the remaining lipids ($C_{6.2}H_{10.4}OS_{0.015}$, 37 MJ/kg). The inventory data for electricity consumption of the supercritical fluid extraction of bio-oil was retrieved from [17,29]. This gives an overall electricity consumption of 10 $kWh/kg_{bio\text{-}oil}$ (cooling, pumping, extraction, separation, and CO_2 heating). The biomass waste is further used to produce biogas and burned in a CHP—combined heat and power unit—thus it could provide electricity and heat to the biorefinery. The biogas composition is 70% CH_4/30% CO_2 by volume, the biogas heating value being 21 MJ/m^3, electrical efficiency 35%, and thermal efficiency 40% [30]. Bio-oil chemical formula was characterized in bench-scale experiments. The nitrogen content of the digestate is obtained by nitrogen mass balance.

Thinking on the potential scale-up of the biorefinery (Figure 6) energy estimates for water circulation, heat, and electricity needs of the batch bioreactor and heat needs of the mesophilic anaerobic digester must be computed. Thus, the energy requirements for the batch reactor in terms of heat ($E_{batch\text{-}heating}$) and electricity ($E_{electricity}$) are estimated as follows. For the heat bath a simplified model is taken from [31], assuming an overall loss factor of 0.5.

$$E_{batch\text{-}heating} = m_{medium} cp \Delta T / 0.5 \quad (1)$$

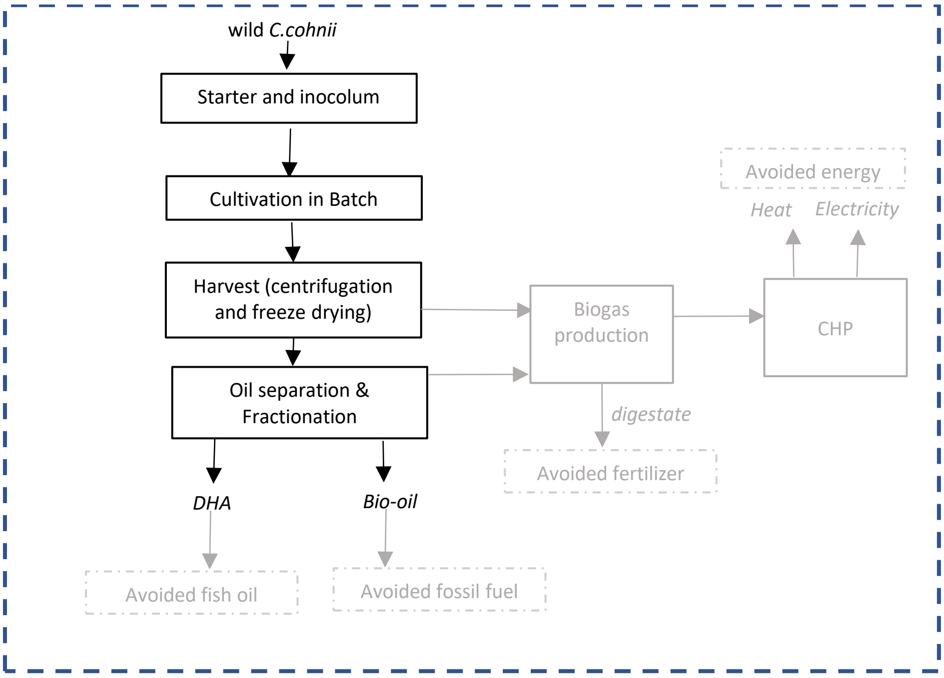


Figure 6. Cascading products scheme.

ΔT is assumed as the difference between 27 °C and ambient temperature of 20 °C. cp is assumed to be 4.18 kJ/kg/K and the medium density as 1000 g/L.

For electricity [32], the Rushton impeller rotational speed (N), the impeller diameter ($Da = 1/3\ D_{reactor}$), the working hours (WH in seconds), the medium density, ρ (by default 1000 g/L), the power number Po (by default 5 for "High shear" impellers [33]), and the electrical motor efficiency (by default η_{motor} 95%, 0.95) are used:

$$E_{electricity} = Po \times \rho \times N^3 \times Da^5 \times WH \times \eta_{motor} \tag{2}$$

The energy requirements for circulating water ($E_{pumping}$) are based on minimum energy requirements to undertake pipe losses and considering an efficiency, η, of 90%.

$$E_{pumping} = m_{h2O} gHL/\eta \tag{3}$$

$$HL = f\ L\ /D\ v\ ^2/2\ g \tag{4}$$

where m_{h2O} is the water mass, HL are the overall losses, f is the friction factor of the pipe taken from the Moody chart (by default 0.04), L is the pipe length in m, D the pipe diameter in m, v the water flow velocity in m/s, and g the gravity constant 9.81 m/s^2.

The heat requirements for the mesophilic anaerobic digester ($E_{mesophilic\ digester\text{-}heating}$) are based on the energy needs to heat the microalgae waste (specific heat from average specific heats for proteins, ash, and assuming an overall loss factor of 0.5).

$$E_{mesophilic\ digester\text{-}heating} = m_{microalgae\ waste} cp_{microalgae\ waste} \Delta T/0.5 \tag{5}$$

$$m_{microalgae\ waste} = m_{dry\ C.\ cohnii} - m_{extracted\ oil} \tag{6}$$

The inventory and mass balances for actual bench-scale data are in Tables 1 and 2. These values are used along with energetic needs assumptions for scale-up to estimate the inventory for 1 ton DHA. Oil without DHA (chemical formula $C_{6.2}H_{10.4}OS_{0.015}$) has H/C similar to that of the usual crude oil-based products (~2) and has O/C of 1/6.2 = 0.16, much higher than the usual crude-oil-based products (<0.05). This means that, at least the oil upgrading step is required to improve oil quality [34]. For example, according to the Dulong formula, oxygen content (m_O) decrease and higher m_H/m_C increases lower heating value (LHV) [35]:

$$LHV\ (MJ/kg) = 33.8\ m_C + 122.3\ (m_H - m_O/8) + 9.4\ m_S \quad (7)$$

Table 1. Inventory for bench-scale.

Unit Process	Inputs	Outputs
Starter	Wild *C. cohnii* Medium	*C. cohnii* starter
Inoculum	*C. cohnii* starter Medium	*C. cohnii* inoculum
Batch (1)	*C. cohnii* inoculum 0.1 (% v/v) Medium 0.9 (% v/v) Incubator (168 h, 50% capacity, 33.6 kWh) Centrifugation (0.5 h, 100% capacity, 0.4 kWh)	*C. cohnii* (77% moisture)
Freeze drying	Vacuum + freezing + heating + condenser 2 kWh/kgH_2O [28] Removed water 0.72 kgH_2O/kg$_{\text{dry algae}}$	70.6 g *C. cohnii* (5% moisture) 0.06 g/L/h (e.g., 3 L 168 h, 70.6 g dry) 27% fatty acids (31% DHA)
Supercritical fluid extraction	Cooling + pumping + heating + extraction + separation 10 kWh/kg$_{\text{bio-oil}}$ [29]	13 g Oil w/o DHA 6 g DHA

(1) Bench-scale data electricity needs based on maximum power equipment and working hours is overestimated up to 10,000 times [32].

Table 2. Mass balances for carbon and nitrogen.

In	Out
70.6 × 0.412 = 29.1 g C	Bio-oil 9.7 g C DHA 4.7 g C Biomass waste by difference 14.6 g C
70.6 × 0.047 = 3.3 g N	Bio-oil 0 g N DHA 0 g N Biomass waste by difference 3.3 g N

Jet fuel specifications require total sulfur content to be less than 0.30 wt.% (ASTM D4294 and ISO 8754) and maritime fuel 0.50 wt.% according to the International Maritime Organization (IMO) limit on ships operating outside designated emission control areas (Regulation 14 of MARPOL Annex VI) with effect from 1 January 2020. Sulfur concentration in oil without DHA (chemical formula $C_{6.2}H_{10.4}OS_{0.015}$) is $0.015 \times 32/(6.2 \times 12 + 10.4 + 1 \times 16 + 0.015 \times 32) = 0.5\%$. This means that a desulfurization procedure must be undertaken for jet fuel compliance. If the electro-deoxygenation process is considered, the electricity consumption is 6600 kWh/300 t biomass and hydrogen consumption 4 g/100 g oil, for 50% oxygen removal [34]. This process removes oxygen, and, for sulfur removal an hydrotreater unit is needed. Energy for catalytic hydrotreating is required to heat the mixed feed and the gas stream to the necessary reaction temperature and to pump and compress the fluids. This unit requires electricity and hydrogen [36]. For electricity the consumption is 3.3 kWh per kg sulfur removed (19,000 btu/bbl, crude 1.26% S, 0.88 kg/L) and hydrogen 1.2 kg H_2 per kg sulfur removed (223,000 btu/bbl, crude 1.26% S, 0.88 kg/L, H_2 120 MJ/kg). There is heat production via exothermal reactions that could enhance district heating potential.

Knowing the volume ratio between CH_4 (x mol) and CO_2 (y mol), and assuming the biogas is only composed by these two gases, one can obtain a system of two unknows and two balance equations:

$$\begin{cases} 12x + 12y = 14.6 \\ \frac{x}{x+y} = 0.7 \end{cases} \quad (8)$$

The solution (x = 0.37, y = 0.85) allows for knowing the biogas moles (1.2 mol) and PTN (100 kPa, 25 °C) volume 1 kmol = 24.8 m^3, representing a $1.2 \times 10^{-3} \times 24.8 = 0.03$ m^3. Therefore, we would expect an electricity production of 0.06 kWh ($0.03 \times 21 \times 0.35/3.6$) and heat production of 0.25 MJ ($0.03 \times 21 \times 0.40$). The nitrogen mass balance allows for estimating the potential of replacing of fossil N fertilizer per mass of dry algae. 0.047 $\mathrm{kgN/kg_{dry\ algae}}$ (3.3/70.6).

2.2. GHG Analysis

To ensure a fair comparison with biorefinery products, the reference, counterfactual, emissions should follow the same assumptions in terms of boundaries. For example, exclude, unless specified otherwise, emissions from capital goods (i.e., manufacture of machinery and equipment, and decommissioning of the power plant and machinery at the end-of-life); emissions due to the transportation and storage; and indirect land use change emissions. Allocation should also follow the same principles when comparing one fossil product with the biobased product.

The reference GHG are computed by multiplying each *C. cohnii* biorefinery output amount by its GHG emission factor (see Appendix A). *C. cohnii* biorefinery GHG is calculated by multiplying the input amounts by their GHG emission factors. Absolute (abs) and relative (rel) GHG savings are as follows:

$$\Delta GHG_{abs} = \text{Reference } [GHG_{abs}] - \text{Biorefinery}[GHG_{abs}] \quad (9)$$

$$\Delta GHG_{rel} = (\text{Reference } [GHG_{abs}] - \text{Biorefinery}[GHGabs])/\text{Reference } [GHG_{abs}] \quad (10)$$

2.2.1. Reference for DHA

Omega-3 fatty acids are proved to be beneficial in various human health disorders [37] and therapies [38]. Most recommendations are for a daily intake in the range of 0.25–0.5 g per person per day [20]. Fish-oil is a major source of long chained omega-3 fatty acids EPA and DHA (eicosapentaenoic (EPA) and docosahexaenoic acid (DHA)), having high market demand in both human nutraceutical industries and animal feed industries [39]. According to historical data, from 1950 until 2010, the top fish taxonomy that provides fish meal and fish oil is Peruvian anchovy [40].

In 2018, about 88% (or over 156 million tonnes) of the 179 million tonnes of total fish production was utilized for direct human consumption, while the remaining 12% (or about 22 million tonnes) was used for non-food purposes. Of the latter, 80% (about 18 million tonnes) was reduced to fishmeal and fish oil [24].

According to FAO data [23,41], roughly 85% of fish captures (from wild and aquaculture) is for human consumption. Fish omega-3 content is on average 387 mg/100 g [42]. Most of the 15% remaining is for fish meal (81%) and fish oil for nutraceuticals (13%). According to FAO, the level of EPA + DHA in fish oil is usually between 15 and 25%

The reduction fishery plant (Figure 7) direct CO_{2eq} emissions are the result of natural gas burning for heat. Indirect emissions are the result of electricity and chemicals inputs. The emission factors used are listed in Table 3. A result of 8.5 gCO_{2eq}/g DHA (mass allocation based) is achieved (see Excel calculations in Supplementary Materials).

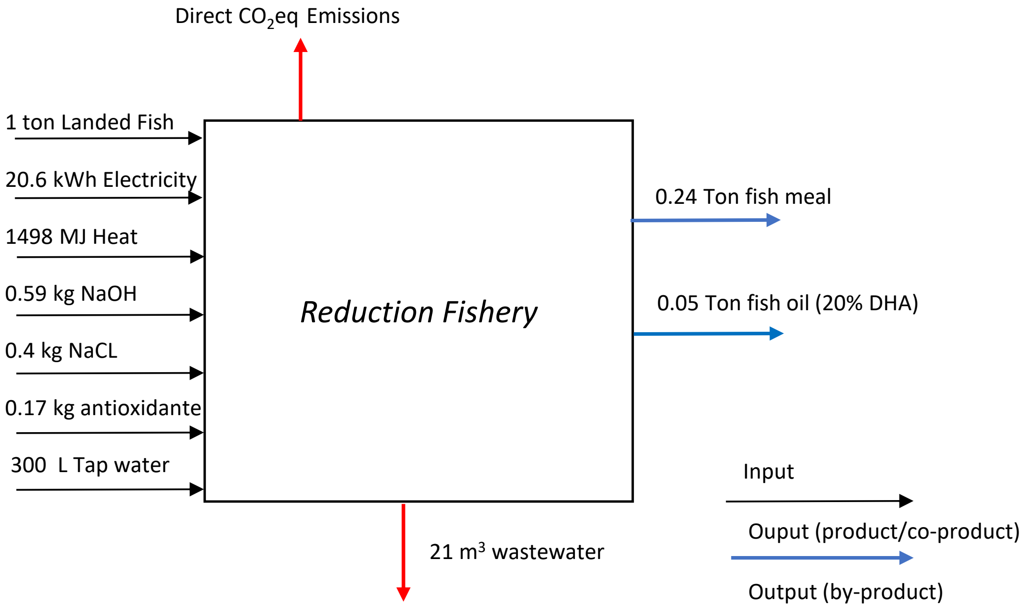


Figure 7. Reduction fishery generic data input and output [43].

Table 3. Selected emission factors.

Item	Unit	gCO_{2eq}/Unit	Database
Heat	MJ	47.3	EU ETS benchmark [12]
Electricity	kWh	230.7	EU27 [44]
Tap water	L	0.149	UK DEFRA [45]
Sodium hydroxide (NaOH)	kg	450 (1) (529.7 × 0.85)	Definition of input data to assess GHG default emissions from biofuels in EU legislation [46]
Sodium chlorine (NaCl)	kg	11.3 (1) (13.3 × 0.85)	
Antioxidants	kg	10,000	[47]
Wastewater	m^3	272	UK DEFRA [45]

(1) Original values have been reduced by 15% to deduct emissions from the extraction and transport of crude oil, NG, etc., as well as transport and distribution of the final fuel that are comprised in REDII but are not accounted for in EU ETS.

2.2.2. Reference for Maritime or Jet Fuel

Refinery energetic consumption is 0.03 kWh/$kg_{crude\ oil}$ and steam 650 kJ/$kg_{crude\ oil}$ [48].

According to the latest Joint Research Centre (JRC) report on the Well-to-Tank analysis [49], the refinery emissions (allocated by product, marginal basis) and its combustion in use: refinery operations cover 7.2 gCO_{2eq}/MJ_{fuel} and combustion in use covers 73.2 gCO_{2eq}/MJ_{fuel}, which translates to an overall emission factor of 80.4 gCO_{2eq}/MJ_{fuel} for diesel fuel. One-hundred-year GWP are calculated using the CO_{2eq} values for CH_4 and N_2O from the Intergovernmental Panel on Climate Change (IPCC-AR5 [50]) (28 and 265, respectively). The summation of these two values excludes transportation, capital goods, and land use. Based on the same boundaries, [46] recommends the value of 78 gCO_{2eq}/MJ for jet fuel and maritime fuel. The baseline emissions for CORSIA (Carbon Offsetting and Reduction Scheme for International Aviation) are 89 gCO_{2eq}/MJ because it encompasses more life cycle steps, and different allocation rules, based on refinery products' energy contents, enlarging the boundaries (see Figure 8). The difference between the two values is 14% ((89 − 78)/78).

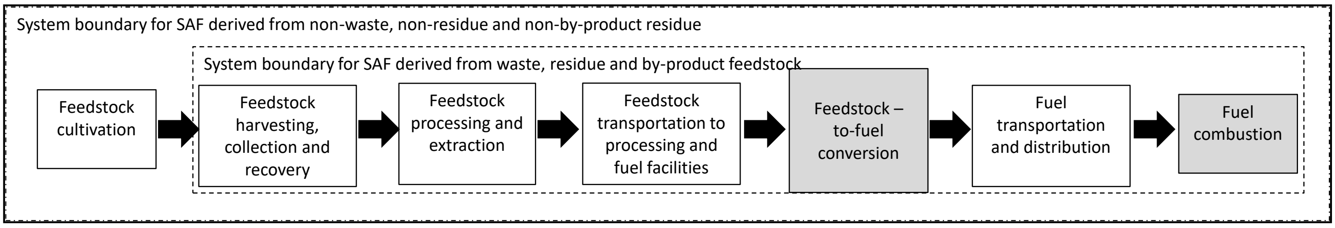


Figure 8. Boundaries in the life-cycle of jet fuel: CORSIA (all) and this study (processes in grey) for the reference scenario.

2.2.3. Reference for N-Fertilizer

Plants obtain carbon, hydrogen, and oxygen directly from water and the atmosphere. Nutrients are also sourced from the environment, with fertilizers being used to supplement those that occur naturally in a given locality, to boost crop yields per hectare. The most used fertilizer is nitrogen (N-fertilizer) that is expected to be in use in roughly constant value of around 20 kgN/capita in 2050, similar to 2010 values [21]. Its industrial production is based on ammonia and natural gas fossil fuel by the Haber–Bosch process. Nitrogen is captured from the air and combined with hydrogen produced from natural gas steam reforming. After the gas mixture is subjected to high pressure (up to 200 bar) and medium-high temperatures (400–500 °C), the exhaust gases are condensed to produce liquid NH_3. The energy requirements are 32 $GJ_{natural\ gas}/t_{NH3}$ [21], 20% electricity, and 80% heat [36]. This translates into an emission factor of 3.89 $kgCO_{2eq}/kgN$, according to the definition of input data to assess GHG default emissions from biofuels in EU legislation and applying a factor of 85% to dismiss transportation and capital goods.

$$12N_2(g) + 32H_2 \leftrightarrow NH_3(g)\ \triangle H = -93\ (kJ/mol) \tag{11}$$

2.2.4. Reference for District Heating

The EU ETS benchmark for heat [12] considers only the emissions of combustion. The natural gas combustion value is 56.1 gCO_{2eq}/MJ, then allocation between combined heat and power units and transportation losses apply. Prior to 2021 this value was set to 62.3 gCO_{2eq}/MJ, but, from 2021 to 2025, according to the Commission Implementing Regulation (EU) 2021/447 of 12 March 2021 determining revised benchmark values for free allocation of emission allowances pursuant to Article 10a(2) of Directive 2003/87/EC of the European Parliament and of the Council, the benchmark value should be 47.3 gCO_{2eq}/MJ (Table 1). By definition, this value excludes transportation and capital goods.

2.2.5. Net-Zero Carbon Electricity

The 26th Conference of the Parties (COP26) of the United Nations Framework Convention on Climate Change was an effort to push and strengthen global ambitions and climate action (SDG13). The International Energy Agency (IEA) produced a report [51] where it explored the way we could reach net zero carbon emissions by 2050. Regarding the electricity sector, at least 90% renewables (photovoltaics, eolic, hydro) must be in place. The remaining 10% would be nuclear, hydrogen, and carbon capture, utilization, and storage (CCUS). For life-cycle values [52,53], including capital goods materials, for renewables a range of 5–217 gCO_{2eq}/kWh (median 45 gCO_{2eq}/kWh) for photovoltaics, 7–56 gCO_{2eq}/kWh (median 24 gCO_{2eq}/kWh) for hydropower, and 3–40 gCO_{2eq}/kWh (median 12 gCO_{2eq}/kWh) for eolic. This means that despite stack emissions being zero, the system is not zero-emissions if the boundaries of the analysis of the system are expanded to cover materials, and, in the case of hydropower, biogenic methane emission from the water reservoirs. Figure 9 shows historical emission intensity from stack (plants operation), life cycle emissions excluding commissioning, maintenance, and end-of-life of the plant. Our hypothesis, for the sake of simplicity, is to assume the boundaries of stack emissions in 2030 (150 gCO_{2eq}/kWh) and 2050 (0 gCO_{2eq}/kWh).

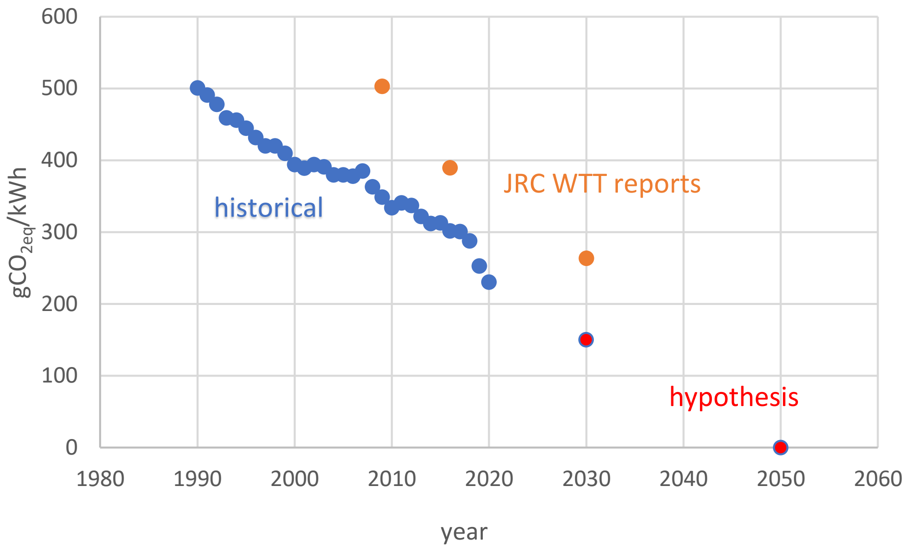


Figure 9. Electricity CO_{2eq} emissions' intensity (historical data from EU27 [44], combustion only); JRC WTT reports data [49] (includes both combustion and upstream emissions occurring before the gates of the power plant and resulting from the production and transport of the fuel, infrastructure materials not included); hypothesis assumed for calculations in 2030 and 2050.

2.2.6. Conventional versus Dynamic Inventory

Throughout the *C. cohnii* biorefinery operation, GHG emission will be released into the air at different time periods, thus causing a different global warming than anticipated by the conventional approach [54,55]. The Python code developed within the research (https://github.com/carlota200/GHG-dynamic-LCA, accessed on 5 January 2022) was used to more realistically cover GHG savings 100 years from the start of the operation.

2.3. TEA Analysis

Capital expenditures (CAPEX) and operational expenditures (OPEX) will be based on three main capital goods: batch reactor, freeze dryer, supercritical fluid extraction, hydrotreating unit, combined heat and power (CHP) unit and the material and energy inputs for 0.14 g/L/h *C. cohni* dry productivity and 27% oil content, production capacity 1 ton DHA/year (see Figure 6). The estimate of capital investment is based on the method of 'Percentage of delivered purchased equipment cost' [56]:

$$\text{CAPEX} = \text{EquipCost}* \sum\nolimits_1^3 fi \tag{12}$$

fi represents a factor for equipment installation, piping, instrumentation and control, electrical systems, building-yard land, service facilities, etc. (f_1 = 3), engineering and supervision, construction expenses, legal expenses, contractors fee, contingency, etc. (f_2 = 1.25) and working capital (f_3 = 0.75).

Yearly updates for equipment capital expenses could be corrected via the Chemical Engineering Plant Cost Index [57]. Equipment cost is based on literature review (Appendix B). For different scale equipment [56]:

$$\text{Cost new/Cost reference} = (\text{Capacity new/Capacity reference})^{0.7} \tag{13}$$

The OPEX (materials and utilities) and products revenue is based on historical commodity prices' lower and higher ends (Figure 10). Maintenance and repair, taxes and insurance, administrative, distribution, marketing expenses, etc., are assumed to be 10% of CAPEX/year, maximum 25% according to [56]. Labor is based on 25 people at 60 k EUR/person/year including overheads [56]. Lower and higher ends of commodity prices (w/o taxes) are taken from several datasets, European Commission [58], Index Mundi [59], Organization for Economic Co-operation and Development and Food and Agriculture Organization (OECD-FAO) [41,60], LIFE public database [61], reports [62–64], and literature survey as presented in [26]. Table A2 summarizes this information (Appendix B). A scenario beneficial to the *C. cohnii* biorefinery will be explored considering the higher end of outputs revenues and lower end for inputs costs.

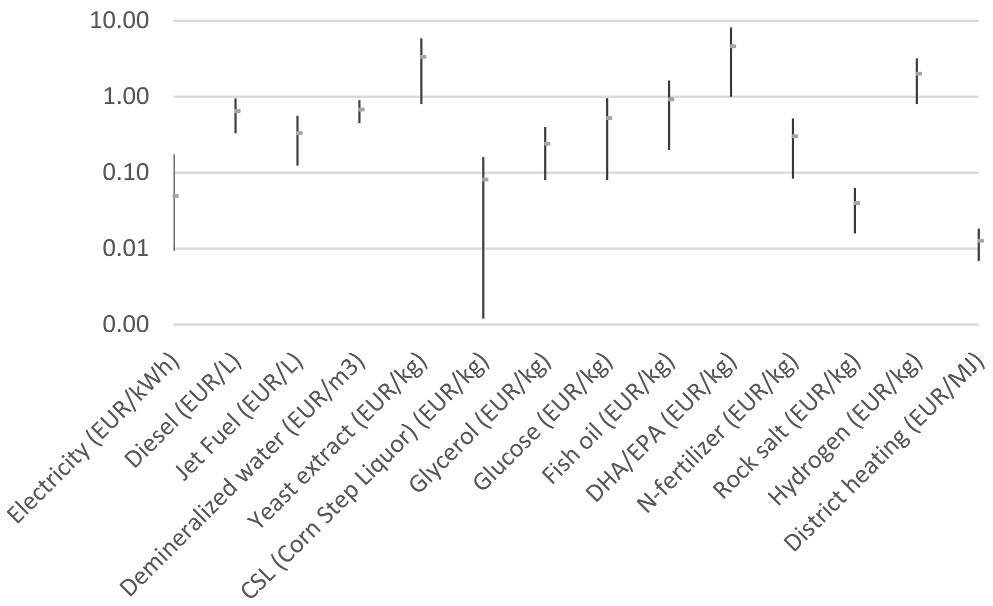


Figure 10. Historical commodity wholesale (EUR 2018) (Appendix B and Supplementary Excel file).

The economic indicators that are used are: the return of investment (ROI) and the inflation-adjusted return (IAR). Operation hours are assumed to be 7200 h/year to ensure maintenance and malfunctions downtimes.

$$\mathrm{ROI} = \frac{REVENUE}{CAPEX} \times 100\% \tag{14}$$

$$\mathrm{IAR} = \frac{1 + ROI}{1 + Inflation\ rate} - 1 \tag{15}$$

The *Inflation rate* is estimated based on the Consumer Price Index (CPI),

$$Inflation\ rate = \frac{(\text{Ending CPI level} \ - \ \text{Beginning CPI level})}{\text{Beginning CPI level}} * 100\% \tag{16}$$

For the sake of simplicity, a linear correlation was obtained from historical European levels (EUROSTAT), having 2015 as reference (Figure 11). Because the inflation rate is a ratio having the same reference it will hold the same results as an absolute-values ratio.

$$\mathrm{CPI} = 1.628 \times \mathrm{year} - 3180.2 \tag{17}$$

Between 2050 and 2030 (and assuming Equation (13)), the accumulated inflation would be 26% which means an average of 1.3% a year.

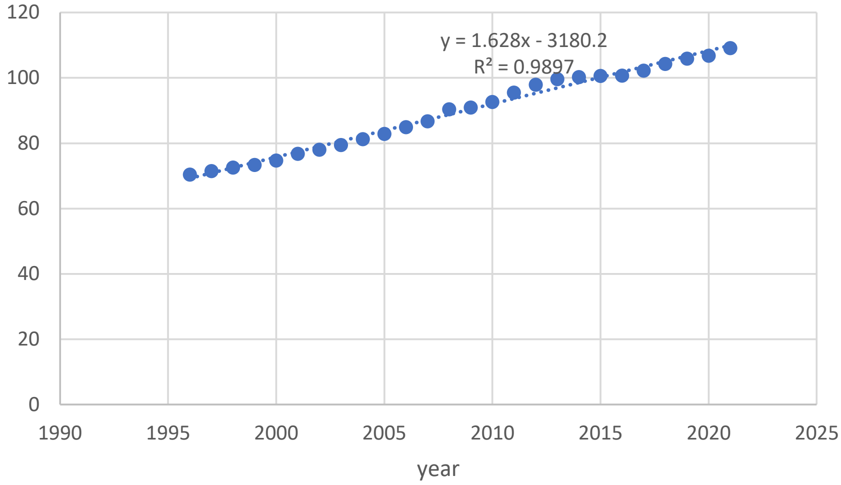


Figure 11. European Union (EU6-1958, EU9-1973, EU10-1981, EU12-1986, EU15-1995, EU25-2004, EU27-2007, EU28-2013, EU27-2020) harmonized CPI (reference 2015) from EUROSTAT.

2.4. Social Analysis

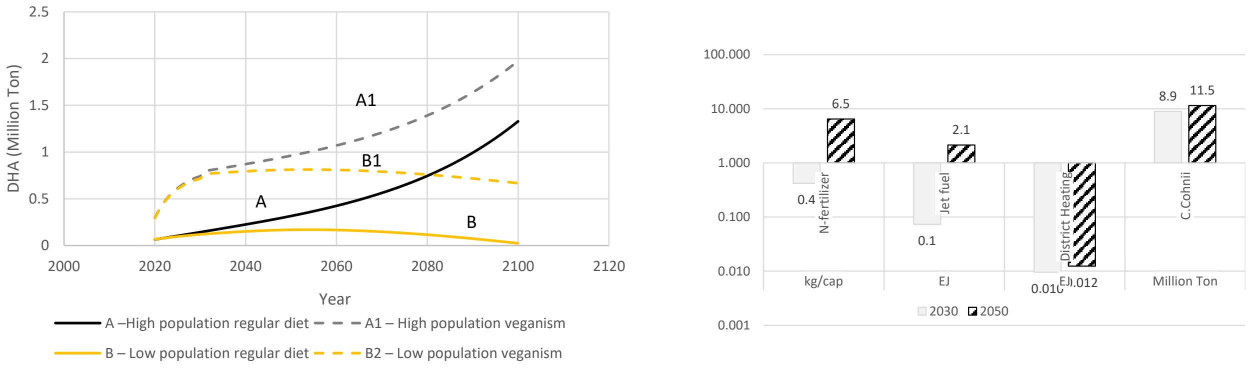
Thanks to a consultancy included in the Portuguese research project OMEGAFUEL PTDC/EAM-AMB/30169/2017, a preliminary and initial exploration of socially relevant aspects of the *C. cohnii* biorefinery concept has been performed, in line with the European framework for Responsible Research and Innovation—RRI: "an approach that anticipates and assesses potential implications and societal expectations with regard to research and innovation, with the aim to foster the design of inclusive and sustainable research and innovation" [65]. The benefits of this new biorefinery concept have thus been considered from a social perspective and areas that need investigation have been identified, to ensure that the technology will meet the interests and demands of societal stakeholders. It is particularly important to do so in the first phases of technology development, to direct research in a way which is desirable for society and to avoid locking society in situations which later on may become difficult to manage [66–68].

3. Results

Results are divided into four aspects: *C. cohnii* future supply needs (high end perspective for maximum levels requirements); biorefinery potential GHG savings in comparison with a reference fossil scenario (reference system, counterfactual); techno-economic assessment for potential investors/stakeholders foster the deploying of the technology; and, finally, each biorefinery product lifecycle value to compare against alternatives and under the sustainability criteria defined for each one if existent. For jet-fuel product, in order to allocate the emissions generated from the entire supply chain amongst all of the valuable outputs of the system, an energy allocation method for co-products is used to be consistent with CORSIA LCA guidelines for jet fuel [69]. Fuel transportation and distribution is by default the average between 0.9 and 0.3 gCO_{2eq}/MJ, i.e., 0.6 gCO_{2eq}/MJ according to CORSIA guidelines. Alternative DHA production from other research are also used for comparison [43]. EU ETS benchmark for heat is used.

3.1. *C. cohnii* Supply Needs

One critical issue, when looking to the biomass potential for replacing fossil pathways, is the necessary feedstock amounts that will meet the products' demands. The product that was chosen as the driver for the demand is DHA. From the experimental data we found a ratio of 0.084 ton DHA/ton *C. cohnii* (see Section 2.1). Based on population projections and fish demand scenarios (see Figures 3 and 4), higher end needs from this kind of biomass assumes that this biomass is the only feedstock available to provide DHA (and this is the reason to call it high end estimates). Figure 12 shows the *C.cohnii* needs supply needs results.



(**a**) (**b**)

Figure 12. (**a**) DHA needs from alternative sources other than fish, (**b**) *C. cohnii* supply needs (higher-end estimates for 100% DHA coverage) and potential coverage of N-fertilizer, jet-fuel, and district heating (0.14 g/L/h *C. cohnii* dry productivity and 27% oil content).

3.2. *C. cohnii* Biorefinery Global Warming Potential and Benefits

Firstly, the inventory for a prospective *C. cohnii* biorefinery is shown in Figure 13. Secondly, the inventory and the GHG emission factor are used in conjunction, for each productivity (g/L/h, dry cell, L of working volume) 0.06–0.4 and oil content 30–70 wt.%. See the Excel file in the Supplementary Materials.

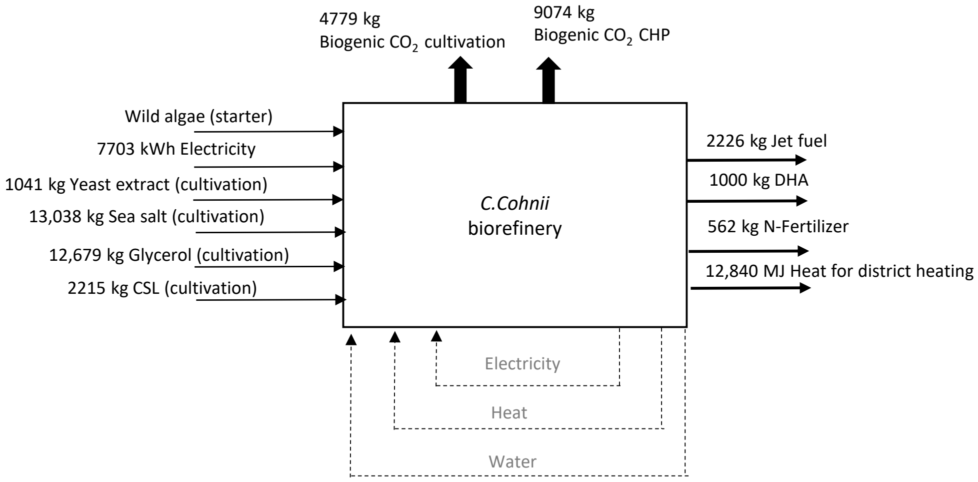
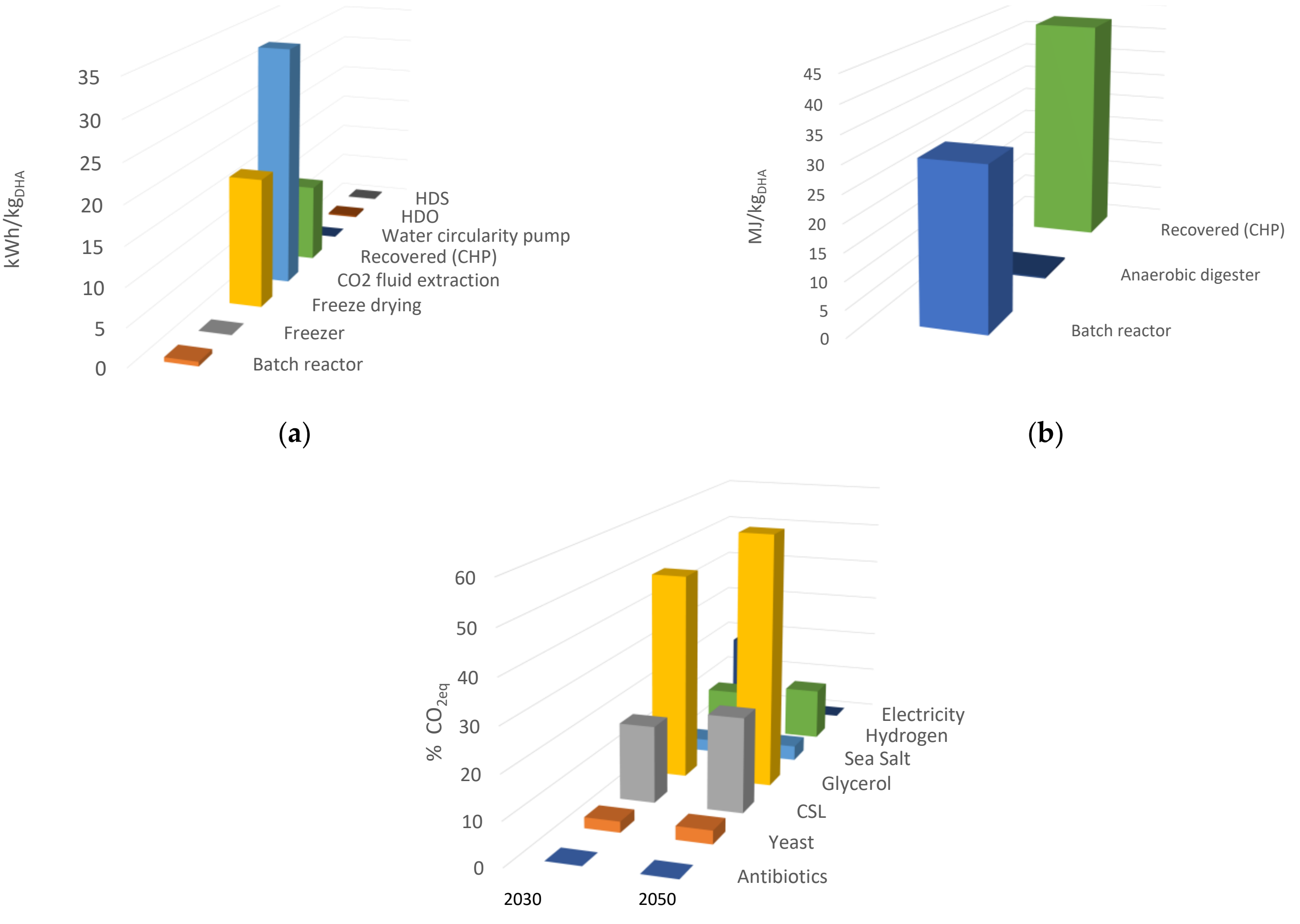


Figure 13. Inventory overview for 1 ton DHA, 0.14 g/L/h *C. cohnii* dry productivity and 30% oil content (used for TEA analysis, see Section 3.4).

The environmental benefits of a *C. cohnii* biorefinery clearly outweigh the reference fossil-based scenario for higher dry cell productivities and oil contents (marked in green Table 4). The main issue preventing achieving 100% savings is the need for hydrogen that is assumed from the current EU ETS benchmark, i.e., produced by natural gas steam reforming. The hotspots in terms of energy are shown in Figure 14, for 0.14 g/L/h *C. cohnii* dry productivity and 27% oil content (*w*/*w*). This value is chosen because it reveals (for the same oil content as lab-scale experiments) higher GHG savings whose values surpass the uncertainty issues related to GHG analysis [70].

Table 4. Matrix of relative GHG emissions savings as a function of average productivity 0.06–0.4 (g/L/h, dry cell, L of working volume) and oil content of 30–70 wt.%. Actual bench-scale data is marked in red [26]. Green values show GHG emission savings higher than 70%.

		Dry *C. cohnii* g/L/h			
		0.06	**0.14**	**0.28**	**0.4**
Oil %	**30**	32	71	84	87
	50	60	81	89	91
	70	70	85	91	92



(**a**) (**b**) (**c**)

Figure 14. Hotspots of the *C. cohnii* biorefinery, (**a**) electricity distribution, (**b**) heat distribution, (**c**) GHG distribution in 2030 and 2050.

3.3. *C. cohnii* Biorefinery Products LCA Values

The resulting GHG emission factors for 0.14 g/L/h and 27% oil content, following the same allocation rules as the reference fossil, mass-based for DHA and N-fertilizer, energy-based for heat and jet fuel in 2030 and in 2050, are shown in Table 5. Noteworthy is the comparison between N-fertilizer 0–1.6 kgCO$_{2eq}$/kgN and the fossil reference of 3.89 kgCO$_{2eq}$/kgN, the comparison between heat 36 gCO$_{2eq}$/MJ and EU ETS benchmark for heat 47.3 gCO$_{2eq}$/MJ, and the comparison between DHA 1.2–1.6 kgCO$_{2eq}$/kg DHA and DHA from a reduction fishery 8.5 kgCO$_{2eq}$/kg DHA.

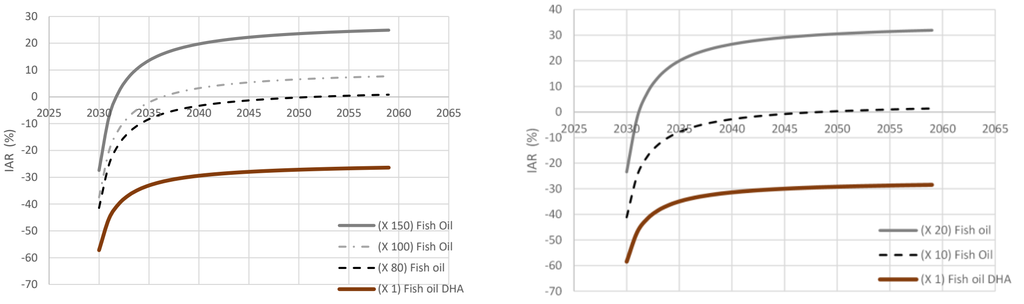
Table 5. Selected emission factors for N-fertilizer, jet fuel, DHA, and district heating from a *C. cohnii* biorefinery.

Item	Unit	2030 gCO_{2eq}/Unit	2050 gCO_{2eq}/Unit	2030 gCO_{2eq}/Unit	2050 gCO_{2eq}/Unit
		Mass Allocation		Energy Allocation	
Heat	MJ	5.9	5.2	35.5	31.0
Jet fuel	MJ	35.9	31.4	35.5	31.0
N-Fertilizer	kg	1585.2	1385.6	0.0	0.0
DHA	kg	1585.2	1385.6	1404.0	1227.2

For jet fuel and according to CORSIA guidelines, the reference value discounting transportation and distribution should be 78 CO_{2eq}/MJ, thus the jet fuel produced from *C. cohnii* allows a potential reduction of, at least, 50% non-biogenic GHG emissions.

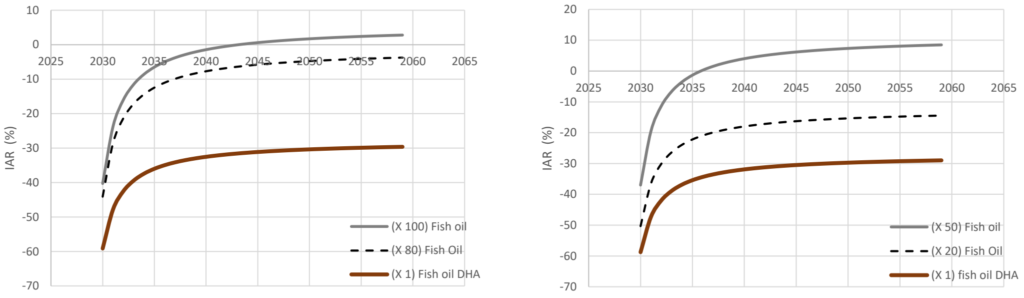
3.4. *C. cohnii* Biorefinery Techno-Economic Analysis

In order to study if DHA obtained from *C. cohnii* biomass can economically compete with fish oil, the best-case scenario is evaluated, giving insight into the best ROI and IAR possible of achieving. The HDS unit can be overlooked if maritime fuel is the targeted crude oil replacer, once the sulfur content of the bio-oil (w/o DHA) is within the IMO limits. The high productivity and oil content scenario (0.4 g/L/h; 70% oil content, Table 5) is evaluated as depicted in Figure 15. These values can be potentially achieved by means of genetic engineering [71]. A more realistic scenario of 0.06 g/L/h and 0.14 g/L/h and 50% oil content without genetic engineering [72] is also depicted in Figure 16. CAPEX and OPEX is shown in Figure 17.



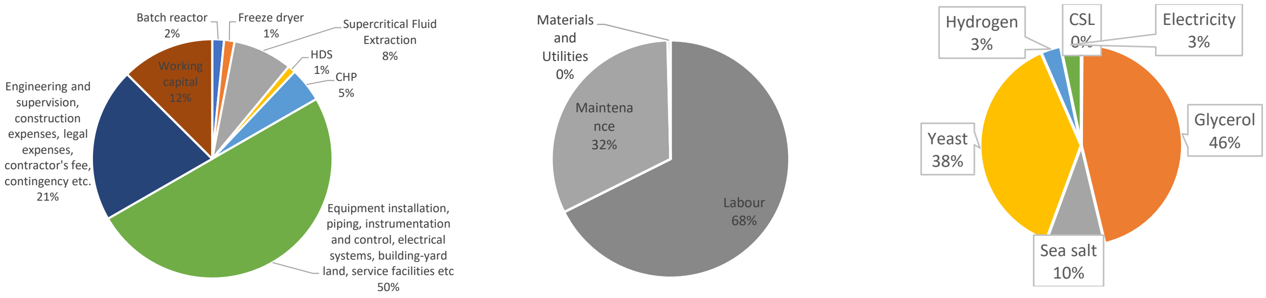
(**a**) (**b**)

Figure 15. IAR economic indicator as a function of fish oil DHA reference cost, (**a**) 0.14 g/L/h and 27% oil content, (**b**) optimized *C. cohnii* 0.4 g/L/h and 70% oil content.



(**a**) (**b**)

Figure 16. IAR economic indicator as a function of fish oil DHA reference cost, (**a**) 0.06 g/L/h and 50% oil content, (**b**) 0.14 g/L/h and 50% oil content.



(**a**) (**b**) (**c**)

Figure 17. CAPEX (**a**), OPEX (**b**), and materials and utilities (**c**) for 0.14 g/L/h and 27 % oil content.

The increase in oil content decreases the carbon and hydrogen content in the *C. cohni* waste and hence compromises the capacity to generate biogas and suppress heat coverage from the CHP unit. Nevertheless the capacity to produce DHA for the same capital goods increases almost 7 times for the same amount of inputs (a result of the ratio of the productivities and oil contents, $0.4 \times 0.7/(0.14 \times 0.3)$). This means that the DHA cost could be more similar to the reference fish oil DHA, in this case 20 times the cost is enough to have a positive IAR after two years of operation.

The economic indicator IAR (Figures 15 and 16) versus GHG savings matrix (Table 4) reveals that the sustainability indicators may be conflicting, namely, if DHA cost is below 10 times the fish oil DHA reference cost for genetic engineered 0.4 g/L/h and 70% oil content (*w*/*w*), below 80 times the fish oil DHA reference cost for 0.14 g/L/h and 27% oil content (*w*/*w*), below 50 times the fish oil DHA reference cost for 0.14 g/L/h and 50% oil content (*w*/*w*), and below 100 for 0.06 g/L/h and 50% oil content (*w*/*w*). In such case, to increase the IAR, either a government incentive is needed, to financial aid or support the *C. cohnii* biorefinery and promote net fossil carbon removals or other co-products' base costs need to be increased in relation to their fossil counterparts.

3.5. *C. cohnii Biorefinery Concept from a Social Point of View*

The *C. conhii* biorefinery presents several characteristics which can potentially be of great interest for society, both at the level of the productive process and of its outcomes. Microalgae biomass is particularly suitable for the extraction and purification of omega-3 compounds, as it is cholesterol free, contaminant free (e.g., heavy metals, polychlorobiphenyls (PCBs)), and tastes good. Algal oil also has other socially desirable attributes, such as its vegetarian and vegan nature, as well as the possibility to be manufactured under kosher or halal conditions [73,74]. Regarding the plant, in the *C. conhii* biorefinery a variety of productive processes can meet and work in synergy (production DHA, maritime/jet fuel, N-fertilizer, and district heating), optimizing processes, and reducing waste and pollution. This could mark a significant improvement in our ability to make good use of natural resources, with a more harmonious relationship between the different phases of the productive process, where nothing is wasted and the value of each element in the process is maximized. Several components of the technology and its development have been selected by researchers to make it ethical and desirable for the consumer and society at large: (i) the production process takes advantage of all microalgal lipid fractions, maximizing the value derived from all fractions, instead of discharging the remaining lipid fraction, after the omega-3 compounds extraction; (ii) microalgae can grow on industrial wastes/residues, such as sugar beet, carob, corn, and rice residues, which is an environmental benefit and simultaneously reduces the production process costs, in terms of feedstock, adding value to biowastes of agri-food industries through biorefinery processing approaches; (iii) the process to separate the omega-3 compounds from the remaining microalgal lipid fraction for biodiesel, using urea complexes and winterization is clean, safe, and environmentally friendly. In addition, the urea complexes can be re-utilized in further separation cycles, which represents an environmental benefit; (iv) the lipid fraction that is intended to be used as biodiesel (composed of alkyl esters), is obtained in the ethyl esters form (using ethanol), instead of methyl esters (which uses methanol, the most used biodiesel composition), since ethanol is less toxic than methanol; (v) all the residues generated in the process are directed to a digestor for biogas production. It is foreseen that the biogas produced can be used as energy source for the process; (vi) the industrial by-product glycerol that is used as carbon source in the medium formulation for the microalgal biomass production, is previously distilled, which means that the toxic contaminants are removed (specially methanol). The microalgae stress response during the cultivations has been evaluated, and we concluded that the stress level is low, indicating that the environment in which the cells were growing was not toxic for them; and (vii) no harmful waste streams are envisaged to land or water. Considering air pollution, the only direct combustion source is the biogas burned at the CHP unit that will not have sulfur, and as an internal combustion engine, could have controlled air pollutant levels.

Since the technology is still in an early phase it will be possible to investigate and check beforehand that the above characteristics fit with the needs of consumers and other societal stakeholders. At the same time, it is important to note that public knowledge is still very low and communication work is required to enable productive exchange with the stakeholders. Another important area to be explored regards which characteristics become relevant at pilot and demonstration stage, especially related to upscaling and implementation at local level. This will ensure that any aspects which could be problematic for social or broad environmental reasons are dealt with, thus that the subsequent development of the technology takes them into account [66]. Based on present knowledge, some points can therefore be proposed for further investigation. Work will be necessary to: (i) understand how the biorefinery would look (and blend in the landscape), how it would use the resources of the territory, if any, and how it could interact with other activities in the area; (ii) test potential consumer satisfaction with the hypothetical products of the C. conhii biorefinery; (iii) involve societal stakeholders to scrutinize the biorefinery concept, also in its possible far reaching effects, and eventually propose improvements; (iv) produce and test end user friendly informative material for societal stakeholders, that could be used to support public awareness and facilitate interaction with local communities with regard to plant planning; (v) identify the most convenient locations for the biorefinery, for technical reasons (for instance, would locations near the sea be preferable?), and for social reasons: which communities could benefit most from hosting such facilities? If there would be any risks or disadvantages at local level with technology at scale; and (vi) verify the legislative and regulatory framework and the conformance of the technology and whether its implementation requires or could be supported by new regulations.

4. Discussion

The transition from a fossil-based economy to a bio-based economy is necessary if an equilibrium is targeted avoiding displacing carbon from the long-term cycle to the short-term carbon cycle. Electrification and electricity generation by 100% renewables is necessary but insufficient in the sense that we still need materials and chemicals, "building blocks", to be produced without fossil contribution.

A prospective *C. cohnii* biorefinery regarding the sustainability related aspects was analyzed, in-line with United Nations Sustainable Development Goals: GHG emission savings from a reference fossil-based system, and the return of investment corrected by the inflation rate (IAR). The physical biorefinery was assumed to have a capacity of 4 ton DHA/year, working hours 7200 h/year, for a batch production of 0.14 g/L/h and oil content 30 wt.%, of which 31 wt.% is DHA. Energy and water circularity were assumed, and extra-heat used as a potential product providing extra income. In this case it was possible to achieve up to 71% GHG emission savings and a positive IAR for DHA cost 100 times the assumed cost of the fish-oil base (or 800 EUR/kg DHA). If a scenario with increased productivity and oil accumulation is considered (0.4 g/L/h and oil content 70 wt. %, 31 wt.% DHA) a cost 20 times the fish oil reference of 8 EUR/kg DHA, 160 EUR/kg DHA ensures an IAR of 20% after 5 years of operation.

Other research projects, PUFACHAIN and ProAlgae, respectively, European and Norwegian Seafood Research Fund—FHF funded projects [75–77]—also reach the conclusion that microalgae based omega-3 needs to have a higher cost than the fish-oil-based. The PUFACHAIN project focused on phototrophic algae. The cost price for DHA was found to be in a range of 700–4000 EUR/kg$_{DHA}$. The ProAlgae project for Spanish and Netherlands case studies found a production cost of 39–135 USD/kg DHA, higher than the fish oil reference of 9.2 USD/kg$_{DHA}$. These values need to be compared with caution due to the different assumptions in the calculations, nevertheless the common insight is that DHA from microalgae is more costly than the fish-oil based.

There is no a unique criterion to assess sustainability of a fuel or material/chemical, which makes it difficult to evaluate alternative products/fuels based on biorefinery in a comparable, fair, and transparent manner. This applies to both GHG analysis and TEA analysis. Focusing on GHG analysis, glycerol is a good example. Some advocate that it should have a zero GHG emission factor because it is a "waste" from biodiesel production. However, in this study it is considered a co-product, a commodity with a market value, and hence with an associated GHG emission factor. Considering 0 kgCO$_{2eq}$/kg instead of 0.2358 kgCO$_{2eq}$/kg, this results in a change from 71 to 86% of GHG savings.

Other aspect can be illustrated with the yeast extract input GHG emission factor. Different datasets can be used for retrieving different values (see Table A1, 0.15 kgCO$_{2eq}$/kg$_{\text{Yeast extract}}$ or 0.617 kgCO$_{2eq}$/kg$_{\text{Yeast extract}}$) and reflecting different assumptions behind the number. The consideration of 0.617 kgCO$_{2eq}$/kg$_{\text{Yeast extract}}$ instead of 0.15 kgCO$_{2eq}$/kg$_{\text{Yeast extract}}$, results in a change from 71 to 68% of GHG savings.

Another aspect usually disregarded is the dynamic nature of the GHG emissions in the atmosphere, for every flux, decaying after a certain time and having different impacts throughout the biorefinery operational years. To gain insight into this aspect, a Phyton model by the grant holder of the Portuguese research project OMEGAFUEL PTDC/EAM-AMB/30169/2017 was utilized. An insight is given considering a constant emission for each year of operation of the biorefinery, for operation lifetimes 30 years and 50 years. Relative GHG savings would be 69% instead of 71% in 2050, and 85% instead of 73% in 2100 (see Supplementary Info). This means that in the long run the *C. cohnii* biorefinery is more beneficial than anticipated by conventional LCA. This analysis introduces an additional level of complexity to the GHG savings but should be regarded as a complementary insight.

Future research directions are highlighted hereafter.

It is important to ensure that the freezing point of the product biojet is lower than −47 °C according to the specification of aviation fuel. Different process evaluations, for example, the replacement of supercritical fluid extraction by a less expensive Soxhlet followed by the separation of both fractions (DHA and bio-oil) using the urea complexation method [78], would be helpful to observe the impact in the GHG and IAR indicators.

The sea salt used in culture media formulation can be replaced by seawater, with some dilution with tap water. Moreover, seawater can provide some trace elements, decreasing the overall cost of the culture medium. This could contribute towards decreasing OPEX, saving chemicals, labor, energy, and freshwater, especially if the future production facility is located near the seaside.

The CAPEX was assumed to be the total capital investment (TCI) in the first year of operation. Some authors consider CAPEX as a linear depreciation of the equipment part of the TCI, direct fixed capital costs, over 15 years lifetime with 8% interest rate and additional taxes of 3.1% [79]. It will be interesting to observe the differences in the cost price for DHA and jet fuel by pursuing this method; nevertheless, it will be quite challenging to consider a specific value and different depreciation rates for different equipment, for the first of a kind biorefinery.

From a social point of view, the concept of the *C. conhii* biorefinery and its productive processes, which appear to be very promising thanks to the characteristics selected up to now by the researchers, still need to be verified in terms of consumers and societal stakeholders' satisfaction. Such a verification, together with investigation of social implications of upscaling and of local implementation of the technology, could form a solid base for further development. However, it must be noted that, given the very innovative nature of the technology, appropriate conditions for the consultation with consumers and societal stakeholders still need to be created, in terms of knowledge, awareness, and relationship with implementers.

5. Conclusions

The aviation, shipping, and chemical industries are doing their own evaluations of biomass availability and sustainability. Sustainability criteria for sustainable fuels and blends in aviation are far more developed than for maritime transport and concentration of the GHG emissions. This means that there is no unique criterion to assess sustainability, which make it difficult to evaluate alternative fuels/products based on a biorefinery in a comparable, fair, and transparent manner. The same applies to the techno-economic assessment where there is no unique set of economic indicators, reference monetary units and year, and percentages of CAPEX as a function of main equipment unit cost.

This research proposes a method to:

- Estimate future biomass feedstock needs (in support of the energy transition);
- Evaluate GHG savings and product GHG emission factors according to both conventional and dynamic LCA, and covering the same boundaries and allocation rules;
- Evaluate TEA based on main units (batch bioreactors, freeze drying, supercritical fluid extraction, CHP, and historically lower and higher commodity cost (prices w/o vat), reference EUR 2018;

Limitations of the analysis are firstly related to the TRL of the experiments, bench-scale 7 L batch reactor, a pilot scale experimental set-up would be preferable (~70 L); heat and electricity balances are estimated, in many occasions, by minimum scale-up requirements which means there is a potential overestimation of district heating offer and electricity demand; the reference fossil based processes do not take into account energetic improvements, for example, regarding electricity net zero by 2050. This later assumption is a way to penalize the reference fossil system because it uses carbon material from geological reservoirs. Likewise, the exclusion of the biogenic CO_2 from *C. cohnii* growth, HDO, and from the combustion of the digestate gas, in the CHP unit, 13.9 $kgCO_2/kg_{DHA}$, favors non-geological carbon sources.

Social, techno-economic, and environmental issues are interconnected, and the resulting features of the technology will make it more or less appealing to society. The concept of the *C. conhii* biorefinery offers solutions that address societal needs both in terms of products and processes. It is therefore well positioned for gaining public support, especially having the possibility to verify at an early stage the interest of societal stakeholders for its development and of being further improved following their input.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/en15103784/s1, Excel files 1 and 2.

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Appendix A

Table A1. GHG emission factors.

Item	Emission Factor	Reference
Aviation kerosene	78.3 gCO_{2eq}/MJ	[46]
Fish oil	1.72 tCO_{2eq}/t	Own study based on [43,80]
Fish oil DHA	8.62 tCO_{2eq}/t	Own study based on [43,80]
N-fertilizer	3886.12 gCO_{2eq}/kg	[46]
District heating	47.3 tCO_{2eq}/TJ	[12]
Antibiotics	6 $kgCO_{2eq}/kg$	[81]
Yeast extract	0.15 $kgCO_{2eq}/kg$	Gabi database *
CSL	0.5 $kgCO_{2eq}/kg$	[82]
Glycerol	0.2358 $kgCO_{2eq}/kg$	[83]
Sea salt	0.0133 $kgCO_{2eq}/kg$	[84]
Hydrogen	6.848 tCO_{2eq}/t	[12]
Electricity	0 or 150 gCO_{2eq}/kWh	Own study based on [46]

* 0.617 $kgCO_{2eq}/kg$ Ecoinvent 2.2 database [85].

Appendix B

Capital costs and commodity wholesale.

Table A2. Commodity wholesale.

Item	Higher End Cost (EUR)	Lower End Cost (EUR)	Reference
Electricity (EUR/kWh)	0.17	0.01	[58]
Diesel (EUR/L)	0.95	0.33	[58]
Jet fuel (EUR/L)	0.56	0.12	[59]
Demineralized water (EUR/m^3)	0.90	0.45	[61]
Yeast extract (EUR/kg)	5.83	0.80	[26]
CSL (corn step liquor) (EUR/kg)	0.16	0.00	[26]
Glycerol (EUR/kg)	0.40	0.08	[26]
Glucose (EUR/kg)	0.96	0.08	[26]
Fish oil (EUR/kg)	1.63	0.20	[41]
DHA/EPA (EUR/kg)	8.17	1.00	Own estimation based on fish oil and 20% Omega-3 content
N-fertilizer (EUR/kg)	0.52	0.08	[60]
Rock salt (EUR/kg)	0.06	0.02	[86]
Hydrogen (EUR/kg)	3.20	0.80	[63]
District heating (EUR/MJ)	0.02	0.01	[62]

Table A3. Capital cost of main *C. cohnii* biorefinery equipment for 4 Ton DHA/year production capacity.

Equipment	Cost (EUR)	Reference
4 × Batch reactor (2000 L) *	4 × 28,077	[87]
Freeze dryer (250 L, 4 shelves, 800 10R vials per cycle)	100,000	[88]
Supercritical fluid extraction (50 L extraction vessel)	567,000	[89]
HDS (small scale)	73,000	[90]
CHP	330,000	[91–93]

* based on 50% working volume.

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