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Process Model and Life Cycle Assessment of Biorefinery Concept Using Agricultural and Industrial Residues for Biohydrogen Production

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Abstract: Sustainable waste management strategies are urgently needed due to an increasing global population and increased waste production. In this context, biorefineries have recently emerged as a promising approach to valorize waste streams and supply a broad range of products. This study presents the process model and life cycle assessment (LCA) of a biorefinery concept using a novel biochemical method, a so-called "dark photosynthesis" conversion. This process is coupled to a photo-fermentation using microalgae. Overall, the biorefinery concept can produce hydrogen, lutein, β-carotene, and proteins for animal feed. Apple pomace from apple juice production is used as feedstock for the primary conversion step. A process model was created with the process simulation software Aspen Plus® using experimental and literature data. Results from this model were then used in an LCA. The environmental impacts of the proposed biorefinery concept are relatively high, showing the need for process optimization in several areas. Energy system integration, stream recycling, and higher hydrogen yields are recognized as especially important for improving the environmental performance of this concept. Despite these findings, the model shows the feasibility of implementing the biochemical conversion technologies in a biorefinery concept for effectively utilizing residue streams.

Keywords: biorefineries; biohydrogen; bio-intelligence; life cycle assessment; process modelling

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1. Introduction

One of the challenges posed by an increasing global population is the corresponding increase in energy consumption. The challenge lies not only in the production and the distribution of energy and energy carriers but also in the de-coupling of energy production and supply from fossil fuels in order to stop climate change. In 2023, global primary energy consumption from fossil fuels amounted to approximately 140 PWh, roughly 81% of total primary energy consumption (172 PWh) [1]. Further, energy demand is expected to reach ca. 247 PWh in 2040 [2]. Therefore, if climate change is to be halted, energy production must become carbon neutral [3,4]. Current geopolitical conflicts also highlight the importance of energy independence, which is strongly linked to the phasing-out of fossil resources.

Hydrogen has recently gained attention as a promising energy carrier for achieving carbon neutrality. It can be combusted or used in a fuel cell to produce electricity, with water as the only by-product. It thus has the potential to contribute to the decarbonization of the energy sector if sourced from renewable resources [5–7]. In particular, hydrogen produced via the processing of biomass, either by applying thermochemical or biochemical methods (biohydrogen), constitutes a scalable and flexible option that can play a crucial role in the transition to a carbon-neutral energy system [5].

Waste generation also increases along with a growing population. In 2016, an estimated 2.01 billion tons of municipal solid waste were generated globally [8]. Under a "business-as-usual" scenario, this number is expected to increase to 2.59 billion tons by 2030 and 3.40 billion tons by 2050 [8]. By 2025, the global waste production is estimated to reach 6 million tons per day [9]. These estimates highlight the need to develop efficient waste management systems to ensure sanitation and the well-being of future societies as well as to prevent ecosystem damage and pollution. Current waste management strategies focus on waste incineration and landfilling, technologies that have negative environmental impacts and are relatively inefficient in terms of energy recovery [10,11]. In 2012, for example, an estimated 1805 PJ was lost to waste incineration and about 1409 PJ to waste disposal in landfills in the EU [12]. In addition, landfilling emits large amounts of GHG, mainly methane, whose warming potential over a 100-year period is 21 times greater than that of CO_2 [13]. Therefore, sustainable waste management strategies must be developed and implemented to manage current and future waste production rates while minimizing environmental impacts. Such strategies must also be consistent with policies and regulatory frameworks targeting sustainable development such as the circular economy and the European Green Deal.

One option for sustainably managing waste is transforming it into valuable products. Historically, anaerobic digestion has been widely used to convert organic waste into value-added products, mainly biogas and fertilizers [14]. It is regarded as one of the most effective processes for waste treatment and energy production [15,16]. The process involves the microbial breakdown of organic matter in the absence of oxygen, leading to the production of biogas, which is mainly composed of methane (CH_4) and CO_2. During digestion, H_2 is produced as a by-product of the decomposition of complex polymers and fatty acids in a series of interlinked reactions. This H_2 is normally converted to CH_4 by methanogenic archaea during the last steps of the digestion process [17]. When using specific microbial consortia and optimized conditions, however, the production of CH_4 can be inhibited, resulting in a product gas with a high H_2 content [18,19]. This approach, however, remains the focus of ongoing research [20].

While anaerobic digestion offers a sound strategy for the valorization of waste, it primarily focuses on the production of energy in the form of biogas. In this regard, the concept of modern biorefineries has recently emerged as a promising strategy for the valorization of waste into a wider range of products, potentially leading to higher economic value [21,22]. The term biorefinery refers to the facilities and processes for the conversion of biomass of all kinds into value-added products like platform and fine chemicals, fuels, and food additives [21,23]. So-called second-generation biorefineries mainly use organic waste as input, thus avoiding competition with food supply and changes in land use. Several conversion methods are available to process biomass into valuable products. Which conversion method is used depends on the type of feedstock used and the desired products.

The valorization and reuse of waste streams in biorefineries contributes to the closing of material cycles, avoiding the extraction of new resources and potentially reducing GHG emissions [21,24,25]. Several studies are available highlighting the potential of biorefineries to valorize residue streams. Some recent examples include a small-scale biorefinery model by Sbarciog et al. transforming wood and food waste into compost, oligosaccharides, fibers, biogas, and fertilizer using various conversion processes in an integrated concept [26]; a pilot-scale biorefinery system by Sarkar et al. producing biohydrogen and volatile fatty acids using acidogenic fermentation of food waste [27]; and a study by Götz et al. comparing the production of 5-hydroxymethylfurfural in a biorefinery concept using miscanthus versus maize as feedstock [28] (see also [29–33]). In addition, if CCS/U technologies are implemented, biorefineries can create CO_2 sinks [34]. Finally, compared to individual technologies for waste valorization, biorefineries can integrate various processes and technologies to optimize the conversion of different types of feedstocks, enhancing overall efficiency and sustainability and maximizing waste utilization [24].

Despite their advantages, the development and implementation of biorefineries has been slow [35]. Uncertainties often hinder process development and full-scale implementation, for example, technology performance, resource availability, return of investments, and food security [35,36]. Furthermore, environmental advantages of biorefineries should not be taken for granted, as an impact reduction in one area, like climate change, does not necessarily mean that overall impacts are low. Therefore, early assessment of the economic, environmental, and social impacts of biorefineries is an important aspect when planning a particular biorefinery concept [35,37].

2. Goal

The goal of this work is to present a process model and an environmental assessment of a new biorefinery concept producing biohydrogen, proteins for animal feed, lutein, and β-carotene using apple pomace from the food industry and an industrial residue stream of ammonium chloride (NH_4Cl). The assessment offers a preliminary evaluation of a newly developed biohydrogen production method called "dark photosynthesis" and its integration with photofermentation. By quantifying environmental impacts and identifying critical process steps, this research supports the development and implementation of the process while addressing existing knowledge gaps regarding the environmental benefits of employing these technologies in biorefinery approaches.

The biorefinery concept is described in Section 3. The life cycle assessment (LCA) methodology was used to estimate environmental impacts based on a process model developed using Aspen Plus® (v 12.0) from Aspen Tech Inc. (Bedford, MA, USA). The applied methodology for these is described in Section 4. Mass balances from the process model and results from the LCA provide an estimate of the potential environmental advantages of producing hydrogen via the integrated biorefinery concept. These are presented and discussed in Section 5. Finally, Section 6 presents the conclusions and briefly discusses potential technological developments.

3. Overview of Biorefinery Concept

Figure 1 shows a schematic overview of the biorefinery concept using the classification scheme proposed by Cherubini et al. [16]. The concept uses an industrial residue stream consisting mainly of solid NH_4Cl and organic residues from the fruit processing industry (apple pomace). Apple pomace is a major by-product of apple juice production with a worldwide generation of several million metric tons [38]. In Germany alone, the production of approximately 400–500 million liters of apple juice per year results in the generation of some 250,000 tons of apple pomace, the utilization of which is far from being fully exploited [39]. It is typically sent to biogas plants or processed into animal feed, which overlooks its potential to produce valuable products through fermentation processes [40]. This underutilization of apple pomace is partly due to its poor shelf life and variable production volumes despite its year-round availability [39]. Its direct use in decentralized and flexible processes therefore has a large potential.

In the biorefinery concept proposed, apple pomace constitutes the main carbon source for a bacterial conversion step known as "dark photosynthesis" [41]. This term describes a novel cultivation strategy using the photosynthetic purple bacterium *Rhodospirilium rubrum*, whereby a high fructose concentration is used to stimulate the photosynthetic apparatus of the bacterium in the absence of light, resulting in the production of H_2 [42–44]. Appropriate pretreatment steps are considered for the feedstock materials, namely fractionation, pressing, filtration, and hygienization for the apple pomace, as well as resuspension for the ammonium chloride stream. The main product of the biorefinery is biohydrogen. The "dark photosynthesis" process uses the purple bacterium *R. rubrum* in a semi-anaerobic batch process, as described by Autenrieth and Ghosh [41]. The gas mixture produced in this step is rich in H_2 and CO_2. It is collected in the reactor's overhead space and directed to a membrane filtration unit where both species are separated. The purified CO_2 is then used as a substrate for a light-dependent photo-fermentation step using the microalgae *Chlorella* *sorokiniana* in an airlift reactor. The biomass resulting from both the "dark photosynthesis" and the algae cultivation steps is further processed to obtain β-carotene, lutein, and proteins for animal feed.

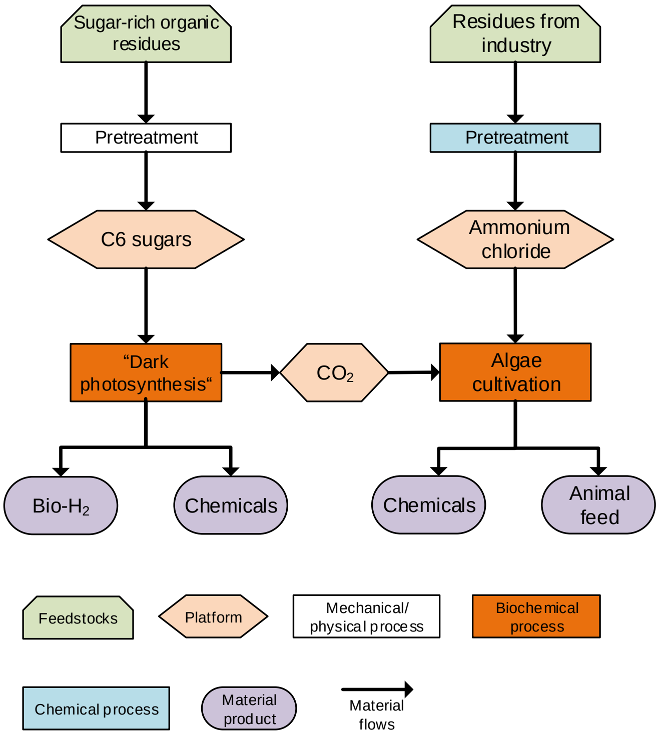

Figure 1. Classification scheme for the model biorefinery concept according to the classification system by Cherubini et al., 2009 [23].

4. Methods

This section is divided in two parts describing the methods used for creating the process model and for carrying out the environmental assessment of the biorefinery concept. An overview of the assessment approach is first discussed. This is summarized in Figure 2.

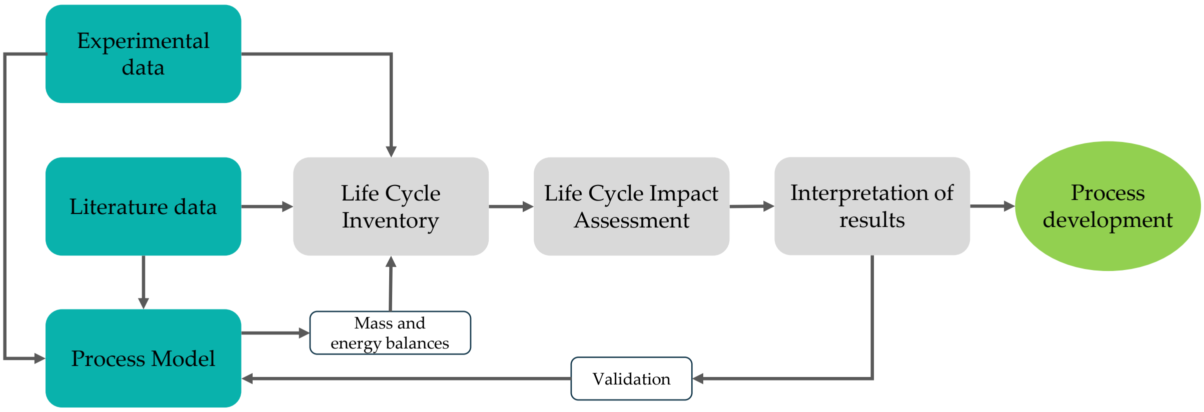

Figure 2. Overview of methodology.

First, suitable system boundaries were defined for the various streams and process chains identified in the biorefinery concept. Subsequently, appropriate indicators were selected according to expert judgement and the literature research on which indicators are most suitable for this type of biorefinery. These include typical LCA indicators like global warming and acidification potentials as well as green chemistry indicators like resource intensity and waste factors. Next, all relevant material and energy flows were identified and integrated into input/output models compiled in Microsoft Excel® (version 2406) spreadsheets. To create these models, laboratory data were obtained from the groups developing the "dark photosynthesis" and algal cultivation steps modelled in this work, namely the Institute of Biomaterials and Biomolecular Systems (IBBS) of the University of Stuttgart and the Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB), respectively. Data on the "dark photosynthesis" step were partially published by Autenrieth et al. [41]. In cases where data were not yet available or could not be extrapolated to the pilot scale, reasonable assumptions were made according to the state-of-the-art and the literature data.

Input and output information were used to create a process model in Aspen Plus®, which in turn served to generate mass and energy balances. These were then used to populate the life cycle inventory for the overall biorefinery concept. Inventory data were integrated into the LCA Software Umberto® (version 11.10.1) from iPoint Systems GmbH and used to calculate the LCA-specific impact categories using the Ecoinvent 3.9 database, while the material and energy balances obtained from the Aspen Plus® model were used to estimate the non-LCA indicators. The main assumptions and parameters for modelling the biorefinery in Aspen Plus® are described in the next section.

4.1. Process Model

The process model was divided into two sections corresponding to the "dark photosynthesis" and the algae cultivation steps. These were modelled separately, as they are de-coupled from each other in the real process and are only connected by hydrogen and CO_2 separation and storage. The "dark photosynthesis" section was modelled as a batch process with a 65 h cycle (Figure 3). The algae cultivation was modelled as a continuous process with CO_2 and NH_4Cl as primary inputs. For both processes, components were defined using the Aspen Plus® databank when available and creating user-defined ("non-conventional") components for the biomass fractions. The non-random two-liquid (NRTL) thermodynamic package was used to model both steps, as both are carried out under ambient pressure. A generic separator block from the unit palette available in Aspen Plus® was used to model the gas separation.

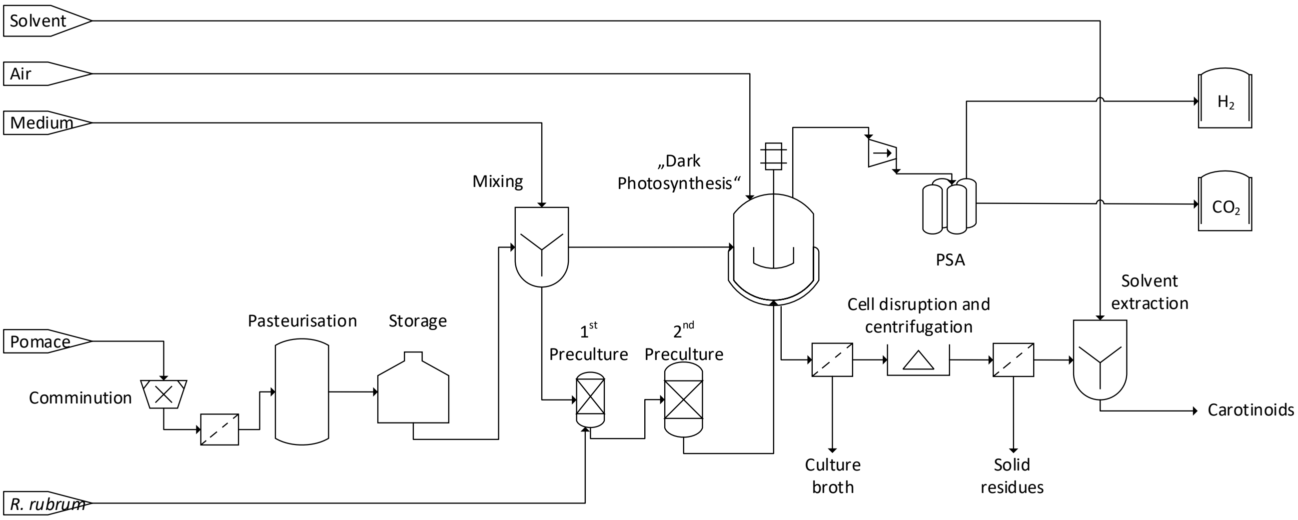

Figure 3. Process diagram of "dark photosynthesis" process step.

In laboratory tests, the amount of fructose that can be effectively extracted from apple pomace was measured as 50 g per kilogram of pomace. Extraction was modelled as shredding, resuspension in water, and filtration, assuming fluid characteristics similar to tomato juice and using generic blocks available in the Aspen Plus® model palette. Electricity consumption was assumed based on similar studies. Apple pomace extract was then subjected to a hygienization step consisting of heating for one hour at 70 °C, as described by Aganovic et al. [45]. Energy requirements were obtained from the heat duties calculated by Aspen Plus®.

The "dark photosynthesis" step was modelled as a batch reaction with a 100 L capacity (60 L working space + 40 L overhead) using one of the predefined reactor models available in Aspen Plus® (RYield). The reactor's temperature was set to 30 °C at atmospheric pressure.

As measured in laboratory-scale tests, a hydrogen yield of 0.25 mol H_2/ mol of fructose per batch was measured [41]. The biomass yield of *R. rubrum* on the M2SF medium measured in these tests was 0.33 g of biomass per gram of fructose. This value is approximately 40% lower than the maximum biomass yield of *R. rubrum* on M2SF medium calculated by Zeiger et al., 2010 [46]. Their study, however, did not focus on hydrogen production and used a fed-batch regime to achieve high-cell density cultures. Therefore, its comparability to the results of this work is limited.

Bacterial biomass was modelled as a non-conventional (i.e., user-defined) compound, using the average cell composition for *R. rubrum* reported by Favier-Teodorescu et al. [47]. Yields and material flows from the carotenoid and protein extraction processes were estimated using the literature data on common solvent/solvent extraction methods using hexane and methanol as solvents (see, for example, the one described by Bóna-Lovász et al., 2013 [48]) along with the biomass yields obtained from process simulations. These steps were modelled as a simple extraction column in Aspen Plus®.

The main inputs to the algae cultivation step are the CO_2 stream from the "dark photosynthesis" and solid ammonium chloride (NH_4Cl). The only pretreatment considered for this stream was mixing with water to a concentration of 0.033%. It is assumed that feeding non-sterile NH_4Cl does not significantly impact the growth of the algae species in the culture, as confirmed by laboratory tests. Feed requirements were obtained from experimental results and used to set the flow rate of both streams.

The continuous algae cultivation using *C. sorokiniana* was simulated using a yield reactor from the reactor palette in Aspen Plus® (RYield). This unit represented the five flat panel airlift photobioreactors of 25 L each where algae are cultivated. An algal biomass gain of 5 gCDW/L per cycle was used (cycle duration = 4 days with an operation time of 24 h per day). Further, an average carbon content of 49% for *C. sorokiniana* was used to estimate the amount of CO_2 fixed per cycle [49]. These values estimated conversion fractions and an overall mass yield fraction for one cycle.

The main parameters used for the Aspen Plus® model of the algae cultivation step are shown in Table 1.

Table 1. Process parameters used for the algae cultivation model in Aspen Plus®.

Component	Value	Unit
NH_4Cl feed rate	3.11	g/h
NH_4Cl conversion fraction	0.756	-
CO_2 feed rate	47.04	g/h
CO_2 conversion fraction	0.249	-
Algal biomass mass yield fraction	0.13	-
NH_4Cl mass yield fraction	0.015	-
CO_2 mass yield fraction	0.704	-

4.2. Environmental Assessment

The environmental assessment of the developed biorefinery concept was carried out using LCA and estimating other environmental performance indicators. This section describes the main aspects of the LCA developed.

4.2.1. Goal and Scope Definition

The aim of the LCA was to determine the environmental impacts of producing hydrogen via the proposed biorefinery concept using a "dark photosynthesis" step combined with a photo-fermentation step using the microalgae *C. sorokiniana*.

4.2.2. System Boundary and Functional Unit

Figure 4 shows the system boundaries defined for the LCA model. According to this boundary, the analysis constitutes a gate-to-gate study. Impacts associated with generating and transporting the NH_4Cl stream were not considered, as this stream is produced on-site. The impacts of apple harvesting and processing are also excluded from the study. This is justified because these impacts occur regardless of implementing the biorefinery concept since only the apple pomace is used as input. Furthermore, the apple pomace is assumed to be produced locally in the vicinity of the plant, so the impacts associated with the transport are assumed to be negligible. Both biological processes, i.e., the "dark photosynthesis" and algae cultivation, are included in the analysis, including all necessary pretreatment steps for the inputs and the production of the culture medium. Co-products of the biological processes are also included in the analysis until they are ready to be transported out of the plant. Impacts from their use phase are thus not considered.

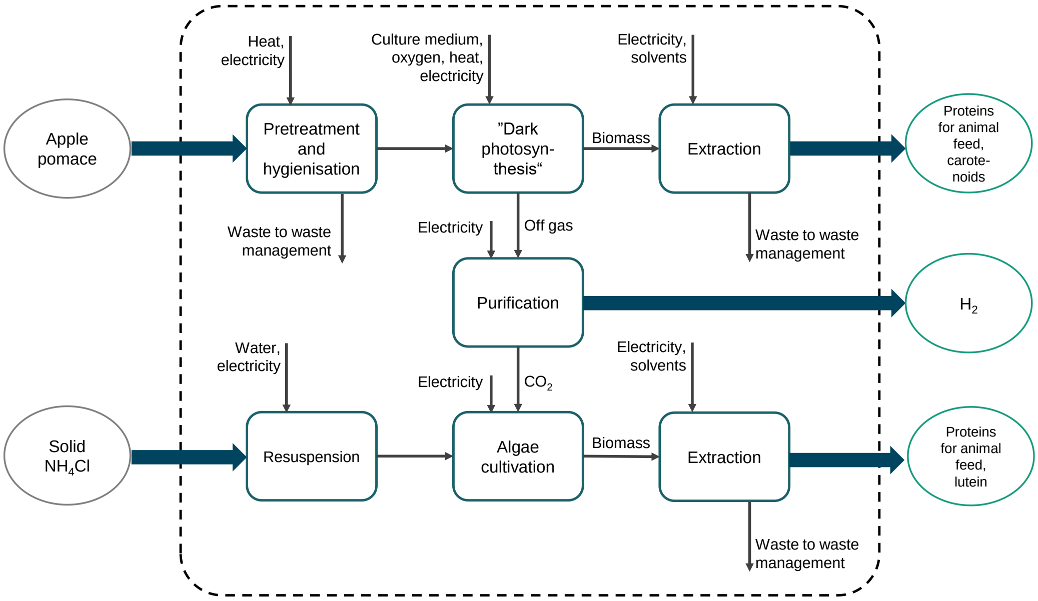

Figure 4. System boundaries of biorefinery concept.

4.2.3. LCA Impact Categories

Five impact categories were selected for the environmental impact assessment of the biorefinery concept: climate change, freshwater and marine eutrophication, freshwater and terrestrial acidification, fossil depletion, and cumulative energy demand. LCI data contributing to these categories are accessible, and the quantification methods for these categories are better established than those of other impact categories. Additionally, these impacts are commonly applied to assess processes and technologies producing fuels and energy carriers [50,51]. Climate change was selected based on its social, political, and environmental relevance, as it is arguably the most pressing environmental issue of our time. Acidification potential is relevant to this process due to the relatively large volume of wastewater produced by the biorefinery concept. This wastewater contains different chemical species that may contribute to an increased acidity. It also reflects the environmental burden of commonly regulated emissions such as NO_x and SO_x compounds. Similarly, eutrophication is relevant due to the potential enrichment of ecosystems with nitrogen compounds from biological processes (specifically NH_4Cl for algae cultivation). Finally, the impact categories fossil depletion and cumulative energy demand were selected because of the relevance of hydrogen as an energy carrier and the focus on substituting a fossil resource. The hydrogen production pathway contemplated in this study does not use a natural fossil feedstock. Quantifying this impact and comparing it to the conventional fossil-based route is thus a relevant indicator for assessing the environmental performance of the concept.

4.2.4. Life Cycle Impact Assessment (LCIA)

The LCIA method used in this study was Environmental Footprint (3.1), with a final weighting (including robustness) approach. Selected indicators of the different impact categories available were chosen. The LCIA was based on primary and secondary data from laboratory-scale tests, mass balances from the AspenPlus® model, data from the Ecoinvent (3.9.1) database, and the literature data. Calculations were carried out based on the integrated biorefinery, taking one batch of "dark photosynthesis" as a reference for calculating the yields of the algae cultivation and the biomass processing steps.

Electricity consumption was modelled as electricity mix from the German grid. Electricity demand for pumps and agitators was obtained from conventional engineering design sources. Heat was modelled as heat for district and industrial uses produced via a conventional power plant using heat and power co-generation. Heat duties were obtained from the Aspen Plus® model.

4.2.5. Other Assessment Criteria

Other assessment criteria complementary to LCA indicators are shown in Table 2 below [52]. The metrics energy intensity, waste factor, and feedstock intensity can be estimated by calculating all material and energy flows within the biorefinery concept. (In this study, this was achieved using the process model developed in Aspen Plus®.) The use of hazardous chemicals is a qualitative indicator referring to the use of substances classified as hazardous under the EU-REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) legislation. It was thus determined by assessing the hazardousness of all materials used in the process.

Table 2. Further non-LCA environmental indicators.

Indicator	Description	Unit
Waste factor	Amount of waste produced for all products and co-products	kg waste/kg product
Feedstock intensity	Mass of feedstock needed to produce all products and co-products	kg of feedstock/kg product
Energy intensity	Energy needed to produce all products and co-products	kWh/kg product
Hazardous chemical use	Qualitative indicator for the use of hazardous chemicals in the production process	Good/Bad

The waste factor describes the amount of waste produced by a given production process. It considers solid, liquid, and gaseous waste. A lower waste factor is preferred, as this indicates that less waste is generated through the production of products. It is calculated according to the following expression:

$$Waste - factor = \frac{M_{Tot.\ W}}{M_{Prod} + M_{co.Prod}}, \tag{1}$$

where $M_{Tot.\ W}$ is the total mass of waste generated from the production process, M_{Prod} is the total mass of the target product generated (in this case hydrogen), and $M_{co.Prod}$ is the total mass of useful co-products generated (in this case proteins for animal feed and β-carotene) [52].

The feedstock intensity metric describes the total amount of key raw material needed to produce a unit mass of the target products and co-products. It provides an overview of the efficiency of the feedstock transformation technology used in the process. A higher feedstock intensity suggests that more raw materials are used to produce a certain amount of product, and thus, the process is less efficient. It is calculated as follows:

$$Feedstock\ intensity = \frac{M_{raw.mat}}{M_{Prod} + M_{co.Prod}}, \quad (2)$$

where $M_{raw.mat}$ is the total amount of feedstock used in the process, and M_{Prod} and $M_{co.Prod}$ have the same definition as above. Similarly, the energy intensity is calculated by dividing the total energy required in the process by the total amount of product. ($M_{raw.mat}$ in Equation (2) is replaced by the total energy consumed in the process.)

The use of hazardous chemicals is a qualitative indicator referring to the use of substances classified as hazardous under the EU-REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) legislation. It was thus determined by assessing the hazardousness of all materials used in the process.

4.2.6. Allocation

Since several products are generated along with hydrogen, environmental impacts must be distributed among them: an approach known as allocation. Various methods can be used to allocate impacts, for example, according to the flow's mass, stoichiometry, or economic value. In general, however, the ISO standard 14044 recommends avoiding the use of allocation by, for example, subdividing or expanding the product system [53]. In the case of biorefineries, allocation is particularly difficult because multifunctional systems can be grouped in several categories and different product categories can be produced in one process (for example, energy, fine chemicals, and fertilizer) [54]. Furthermore, choosing which product is displaced by the biorefinery's output is often difficult and can produce misleading results. Therefore, the allocation method should be assessed on a case-by-case basis according to the specific context of the biorefinery assessed, and results should be reported as transparently as possible.

In this study, a mass allocation was applied among all products of the biorefinery concept (hydrogen, lutein, carotenoids, and protein for animal feed).

5. Results and Discussion

5.1. Process Model

The mass and energy balances carried out in Aspen Plus® showed that the biorefinery concept yields 0.74 kg of lutein, 0.23 kg of β-carotene, and 130 kg of animal feed per kilogram of hydrogen produced. For this, 4.9 tons of unprocessed apple pomace are needed. In this case, however, energy consumption in the form of electricity is exceedingly high (above 10 MWh), given the low hydrogen yield per batch and the high energy requirement of the membrane separation system, as modelled.

Table 3 below shows the primary material flows for the "dark photosynthesis: step. Protein and β-carotene content was determined via laboratory tests using the same cultivation conditions as used for the process model (β-carotene and protein content of 2% and 54%, respectively).

Table 3. Material yields for the "dark photosynthesis" step.

Component	Value	Unit
H_2	0.25 2.80×10^{-3}	mol/mol fructose g/g fructose
Biomass	0.33	g/g fructose
Protein for animal feed	0.18	g/g fructose
β-carotene	6.64×10^{-3}	g/g fructose

Table 4 below shows the calculated material yields for the algae cultivation step. The main products of this step are lutein and protein for animal feed. These were calculated assuming a biomass concentration of 0.5% and 40% by weight, respectively. Total product refers to the sum of the total lutein and protein produced.

Table 4. Total material yields of algae cultivation step for one cycle (one cycle = 4 days, 24 h/day operation; total reactor volume = 125 L).

Component	Value	Unit
CO_2 fixed	1.13	kg
NH_4Cl used	85.1	g
Algal biomass	737	g
Lutein	3.69	g
Protein for animal feed	295	g

The model developed within this work successfully implements the available literature and empirical data into a digital representation of the overall process and estimates material flows and product yields. These estimates are based on preliminary laboratory data but can still help guide process development. At this stage of the process development, however, the applicability of the developed model remains limited. Limitations are mainly attributed to the lack of experimental data that can be used to accurately model the relevant reactions. Efforts on optimizing the *R. rubrum* strain for hydrogen production are ongoing, so yields are expected to increase. Moreover, the model does not account for discrepancies between the laboratory- and the pilot-scale processes in terms of reaction kinetics and up-scaling effects. Since the data used to calculate reaction rates and kinetics were generated in laboratory-scale tests, results for the full-scale process may differ significantly. This problem is particularly relevant for the "dark photosynthesis" process because it is carried out in batch mode. At a larger scale, issues such as limitations in the oxygen transfer rate, slow nutrient uptake, and the development of temperature profiles in the reactor may become more prominent, leading to deviations from the predicted behavior. Finally, purification and extraction processes were not modelled with process simulations but rather estimated from the literature data. These constitute a significant uncertainty in the estimation of the selected metrics.

Important limitations also exist in the modelling of downstream process units. This model simplifies the purification of hydrogen gas to offer an estimate of the process yields without considering the kinetics involved in the process. This can be reflected in the calculated environmental and economic impacts, for example, if the membranes used are expensive or if the overall separation is inefficient.

5.2. Life Cycle Assessment

The evaluation carried out in this study showed that the biorefinery concept generates 24.4 kg CO_2-eq. per kilogram of hydrogen produced, of which 0.172 kg CO_2-eq. is attributed to biogenic carbon. This is almost twice the emissions of conventional fossil-based hydrogen produced via steam reforming of natural gas (12.3 kg CO_2-eq. per kilogram of hydrogen) as calculated by Patel et al. [55]. The total energy requirement of the biorefinery concept is 419 MJ per kilogram of hydrogen, more than three-fold the lower heating value of hydrogen (120 MJ/kg). This suggests that, as modelled, the biorefinery system is not more environmentally friendly than the fossil-based alternative (grey hydrogen), at least when considering hydrogen alone.

Table 5 below shows the calculated impacts for the assessed impact categories for producing 1 kg of hydrogen gas. A time horizon of 100 years is assumed. Mass allocation over the entire process was used to account for the different products generated in the biorefinery concept. In this case, no credits were assigned to the produced co-products (lutein, β-carotene, and protein for animal feed).

Table 5. Calculated environmental impacts for 1 kg of hydrogen using mass allocation. GWP = global warming potential.

Impact Category	Value	Unit
GWP (fossil)	24.4	kg CO_2-eq
Freshwater eutrophication	0.036	kg P-eq
Marine eutrophication	0.020	kg N-eq
Freshwater and terrestrial acidification	0.180	mol H^+-eq
Fossil depletion	351.2	MJ
Cumulative energy demand	419.4	MJ

The assessment of these impacts was divided according to the main stages in the process: (1) raw materials, (2) pretreatment, (3) "dark photosynthesis", (4) algae cultivation, (5) gas purification, and (6) biomass processing. An overview of the contribution of each process step to the respective impact category is shown in Figure 5. Most of the impacts occur in the first three process steps (raw materials, pretreatment, and "dark photosynthesis"). For the impact indicators global warming potential (GWP) and marine eutrophication (ME), around 80% of the environmental impacts are caused during the conversion steps. A further 10–15% result from the choice of raw materials and 8–10% from the pretreatment steps. Figure 4 shows the normalized impacts for all impact categories.

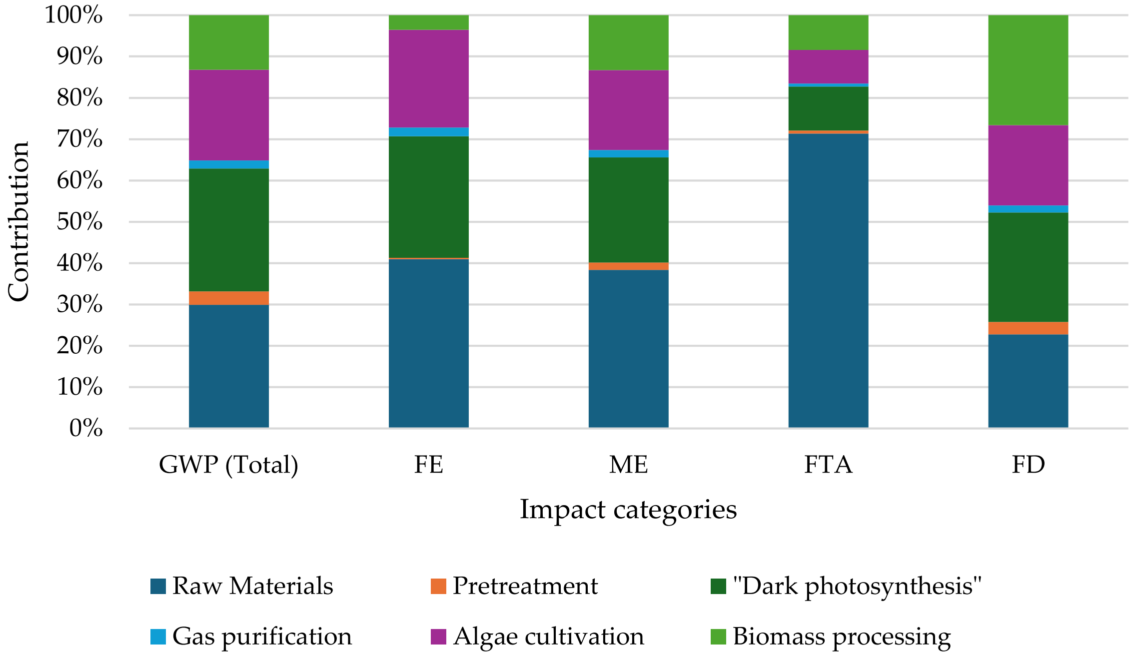

Figure 5. Process contributions to LCA impact categories (GWP = global warming potential; FE = freshwater eutrophication; ME = marine eutrophication; FTA = freshwater and terrestrial acidification; FD = fossil depletion).

The "dark photosynthesis" step is mainly responsible for the environmental impacts in almost all categories. Most of these impacts are due to this step's electricity and heat requirements, such as pumping and maintaining the cultivation temperature. Raw materials have a slightly higher contribution only for the impact category acidification potential. These impacts come mainly from the production of nutrients for the M2SF culture medium. Pretreatment of feedstocks has a minor impact on the selected impact categories despite the relatively high electricity and heat demand for the hygienization of the used feedstock.

Like for several LCA studies, the topic of impact allocation is the subject of debate [56]. The difficulty therein lies in the selection of an appropriate allocation method (e.g., mass or economic value basis) that accounts for the wide spectrum of products produced in a biorefinery. In the case of the biorefinery concept presented in this study, for example, different types of products are produced in varying amounts and with significantly different uses. This poses a challenge when deciding on how impacts should be allocated to the different products generated [54].

The estimated GWP for the "dark photosynthesis" step is significantly higher than those reported for other biotechnological processes for biohydrogen production. For example, Manish and Banerjee [57] calculated emissions of a dark fermentation process coupled with a photo-fermentation using sugarcane juice as main carbon source. They estimated emissions of 3.4 kg CO_2-eq./kg bio-H_2 when by-products were not considered. Similarly, Djomo et al. [58] calculated a GWP of 1.25 kg CO_2-eq/kg of hydrogen for a similar process using potato steam peels as substrate. Both studies considered different allocation approaches for attributing impacts to hydrogen and co-products. Djomo et al., for example, considered the amount of gasoline fuel and animal fodder displaced through the production of hydrogen and proteins for animal feed [58]. These "avoided" impacts were credited to their products, lowering their impacts. Furthermore, different process configurations were considered, including some with optimized energy utilization and stream recycling.

Several factors contribute to the high environmental impacts of the studied biorefinery concept. Firstly, design options aiming at improving energy and resource efficiency were not considered, for example, implementing recycling streams and energy recovery steps in the form of heat exchangers. Further, alternative energy sources with lower environmental impacts could be considered, including using renewable electricity and heat or using residue streams to produce heat. Such strategies must be assessed in other studies, also considering economic and performance aspects. Secondly, as mentioned above, product yields are based first on laboratory-scale results using non-optimized *R. rubrum* strains. Optimization of the bacterial strains used could result in better yields, in turn reducing impacts per unit of product produced. Finally, energy requirements are typically a function of process scale and cannot be directly extrapolated from pilot-scale data. Scaling up the process can lead to lower energy efficiency due to differences in equipment size and process integration, thus increasing the environmental impact associated with energy use. This is particularly important for the "dark photosynthesis" system, which has the highest energy consumption. On the other hand, process optimization and enhanced temperature management can counter these effects. These aspects will be considered in future research to assess the sustainability of a full-scale facility incorporating the technologies described.

Table 6 shows an overview of the impacts of similar biorefinery concepts reported in the literature. These use different conversion platforms and feedstocks from the ones used in this study. Concepts using coupled dark fermentation and photofermentation showed 4- to 5-fold lower CO_2-eq. emissions than those calculated in this study. Similar results can be found for gasification and steam methane reforming of biogas. However, a direct comparison is difficult, mainly due to the differences in scope and methodological assumptions of the studies presented. The latter refer mainly to different allocation approaches used for assigning impacts to all products generated in the biorefinery. Nevertheless, impacts from this study are significantly higher, especially considering the reduced scope considered (gate-to-gate). There is potential for optimization of "dark photosynthesis"-based concepts, particularly in terms of yield and energy recovery (see next section), which could lead to climate change impacts in comparable to those reported in the literature.

To validate the results obtained, further assessment is needed. For example, other impact categories could be measured. In addition to the ecological impacts, an economic study can expand current knowledge of the biorefinery concept and enable a more comprehensive assessment. Furthermore, process data from pilot-scale tests are needed to validate the results obtained in this study.

Table 6. Overview of reported GWP impacts for different conversion platforms and feedstocks. DP = "dark photosynthesis"; DF = dark fermentation; PF = photofermentation; AD = anaerobic digestion; SMR = steam methane reforming.

Reference	Feedstock	Conversion Platform	GWP (kg CO_2-eq/kg H_2)	Remarks
This study	Apple pomace	DP + PF	24.4	Gate-to-gate scope. No credits assigned for co-products
Zech et al. [59]	Biomass (not specified)	DF	5.28	Considered hydrogen distribution
Reaño [60]	Rice husk	DF	10.92	Gate-to-gate scope
Djomo et al. [61]	Steam potato peels	DF + PF	5.18	Considered use phase of hydrogen in transport sector. Results do not consider co-products (animal feed)
Djomo et al. [61]	Sweet sorghum stalk	DF + PF	5.32	Considered use phase of hydrogen in transport sector. Results do not consider co-products (animal feed)
Djomo et al. [61]	Wheat straw	DF + PF	5.6	Considered use phase of hydrogen in transport sector. Results do not consider co-products (animal feed)
Hajjaji et al. [62]	Organic waste (not specified)	AD + SMR	5.59	Accounted for avoided emissions from fertilizer production (co-product)
Chen et al. [63]	Lignocellulosic waste	Gasification	4.41	Cradle-to-grave scope. The concept uses solar energy to cover electricity demand
Reaño [60]	Rice husk	Gasification	20.1	Gate-to-gate scope
Zech et al. [59]	Biomass (not specified)	Gasification	4.08	Considered hydrogen distribution

5.3. Sensitivity Analysis

A sensitivity analysis was carried out to evaluate the robustness of the LCA and determine the influence of selected process parameters on the GWP impact category. This impact category was preferred due to its current relevance. The process parameters that were varied were the H_2 and CO_2 yields and the heat and electricity requirements of the "dark photosynthesis" step. The yield parameter was selected because it constitutes the target of ongoing process optimization efforts, focusing on increasing H_2 yields through genetic modification of *R. rubrum* strains and bioprocess engineering approaches. Similarly, heat and energy requirements can be reduced through various strategies like energy recovery on-site, process integration, and optimization of energy utilization. When upscaling the process, however, energy and heat requirements might increase non-linearly so that an increase in the GWP indicator is possible.

Figure 6 shows the results of the sensitivity analysis. As expected, the H_2 yield has the greatest influence on the GWP indicator, followed by the electricity and heat demands, respectively. These results suggest that a large reduction in GWP can be achieved if H_2 yields of the "dark photosynthesis" step are improved. With a measured yield of 0.25 mol H_2 per mol of fructose, yields are significantly low compared to those reported for dark fermentation, which are usually above 1 mol H_2 per mole of sugar (see, for example, references [57,60,64,65]). Increasing yields to 0.6 mol H_2 per mol of fructose, for example, would lead to a reduction of approximately 58% in the GWP impact category. Such yields were measured in laboratory tests using pure fructose as substrate, suggesting that such a scenario is at least plausible. One major challenge, however, is achieving higher fructose concentrations in the culture medium without increasing energy demand through, for example, evaporating the resuspended apple pomace. Further optimization of H_2 yields is the focus of ongoing research.

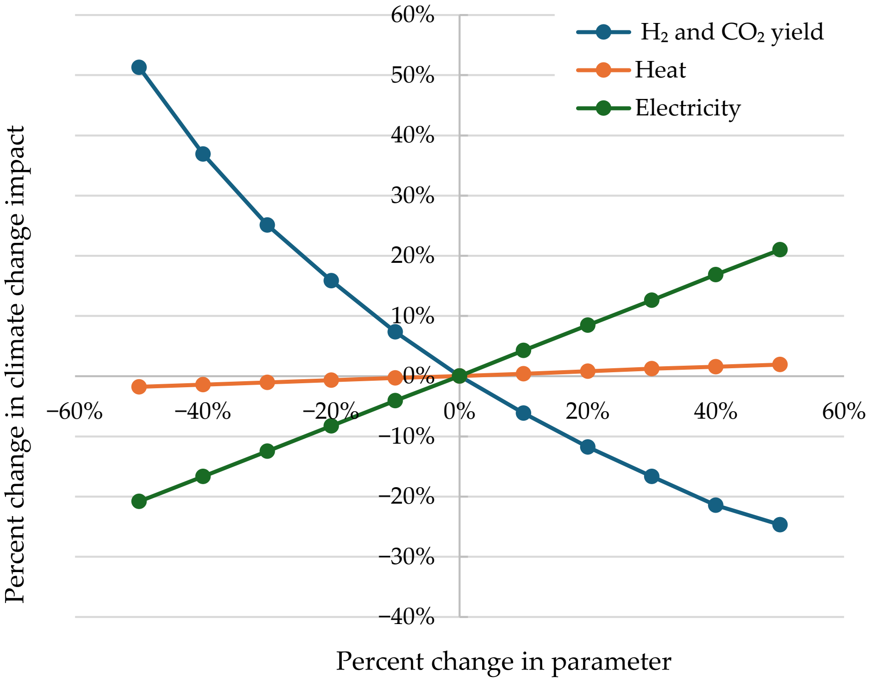

Figure 6. Sensitivity analysis showing the percent change in the GWP impact category resulting from a change in selected parameters of the "dark photosynthesis" step.

Strategies for improving energy efficiency include energy recovery from process residues and integration with other processes on-site. Similar scenarios have been considered in several studies, showing important reductions in total impacts when co-generation of heat and electricity are considered (see, for example, [64–67]).

5.4. Other Assessment Criteria

Table 7 below separately shows the calculated non-LCA criteria for the "dark photosynthesis" and algae cultivation steps. In this case, the indicators were calculated using the total weight of products generated in each step instead of a single functional unit. Feedstock efficiency was calculated by adding the weight of all input materials for each conversion step and dividing by the total weight of products generated.

Table 7. Non-LCA assessment criteria for overall biorefinery concept.

Indicator	Value	Unit
Waste factor	2.13	kg waste/kg product
Feedstock intensity	36.9	kg feedstock/kg product
Energy intensity	176.0	kWh/kg product
Use of hazardous chemicals	Potentially bad, depending on extraction method used [1]	

[1] According to the European Chemicals Agency's C&L Inventory entry for n-hexane, this substance is suspected to be reprotoxic. Extraction with n-hexane can be substituted by other extraction methods such as supercritical-CO_2 extraction.

In general, the estimated impacts are higher for the "dark photosynthesis" step than for the algae cultivation. This is partly because of the batch operation mode used when modelling the "dark photosynthesis" compared to the semi-continuous mode of the algae cultivation system. This translates into a higher feedstock and energy intensity and larger amounts of waste per mass of product produced. This is typical for batch processes, in which productivities per unit time are significantly lower than in continuous processes [68]. It should be noted that most of the waste generated consists of culture medium and rest biomass, both of which are relatively benign in terms of environmental impacts. Further, some of the wastewater produced could be recycled, potentially lowering impacts.

The waste factor, feedstock intensity, and use of hazardous chemicals metrics are useful for assessing biorefinery concepts without the need to conduct a detailed LCA. However, some of the same limitations as for the general assessment of biorefinery concepts also apply to the use of these indicators. For example, results may not adequately reflect the product spectrum produced by a given biorefinery concept or may be misleading when applied to concepts yielding different types of products (energy carriers, fine chemicals, bulk chemicals, etc.). Therefore, results from such metrics must be carefully interpreted and considered in a case-by-case model, considering the context of the biorefinery assessed.

6. Conclusions and Outlook

This study constitutes a first approach towards assessing a novel biorefinery concept based on the “dark fermentation” technology. Results show that the proposed biorefinery concept can effectively use waste streams to produce hydrogen and other products. The estimated product yields, however, are comparatively low, as can be confirmed when comparing results with the literature estimates. This is mainly because the experimental data used for the models correspond to a non-optimized process, where, for example, substrate utilization by the used bacterial and algal strains is not optimal, and only limited data are available. The metrics calculated using results from the process simulations were typical for the type of process used despite limitations in the availability of empirical data. Since these data were based on laboratory-scale results, the assumptions might not hold for the full-scale process. Further, processes were assessed separately without accounting for the overall operation of the concept, limiting the validity of the conclusions derived.

Further development of the “dark photosynthesis” technology, for example, in terms of biohydrogen yields or energy efficiency, can significantly reduce environmental impacts. Future work will focus on optimizing *R. rubrum* strains for hydrogen production using different fructose-rich feedstock. Optimized cultivation strategies like fed-batch cultivation will also be investigated.

The process model created within this work could further be used as the basis for a digital twin of the biorefinery concept. Digital twins can provide an effective process optimization strategy and minimize environmental impacts by targeting efficient substrate use. Their applicability in process control and optimization, especially in the context of integrated biorefineries, is thus large.

Waste biorefineries can make a significant contribution to the establishment of a bioeconomy. When coupled with simulation tools, their design and implementation can be accelerated, resulting in lower environmental impacts, more secure investments, and shorter deployment times. Biorefinery concepts like those described in this work can also contribute to developing green and sustainable chemistry approaches. Chemical products produced in biorefineries constitute a green alternative to products currently produced from the refining of fossil fuels or new products altogether. Thus, biorefineries also contribute to the expansion of product portfolios based on the valorization of decentralized waste streams. This constitutes a significant contribution to the development of more sustainable waste management strategies and a bioeconomy. When substituting products derived from fossil fuels, biorefineries can also make an essential contribution to mitigating GHG emissions.

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