Advanced Metabolic Engineering Approaches and Renewable Energy to Improve Environmental Benefits of Algal Biofuels: LCA of Large-Scale Biobutanol Production with Cyanobacteria *Synechocystis PCC6803*

Daniela Villacreses-Freire (danivill86@gmail.com)

Karlsruhe Institute of Technology: Karlsruher Institut fur Technologie https://orcid.org/0000-0003-2471-9426

Franziska Ketzer

Karlsruhe Institute of Technology: Karlsruher Institut fur Technologie

Christine Rösch

Karlsruhe Institute of Technology: Karlsruher Institut fur Technologie

---

Research Article

Keywords: Life cycle assessment, upscaling, cyanobacteria, biobutanol, scenario approach

Posted Date: June 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-564074/v1

Version of Record: A version of this preprint was published at BioEnergy Research on October 13th, 2021. See the published version at https://doi.org/10.1007/s12155-021-10323-y.

Abstract

Microalgae have the potential to serve as sustainable biocatalysts for direct sun-to-bioproduct approaches, e.g. for the synthesis of biofuels. Genetic engineered mutants with a higher photon conversion efficiency of sunlight into biofuels of interest are capable to serve as powerful green cell factories. These advanced metabolic engineering approaches are expected to have environmental benefits, especially for climate protection.

The Life Cycle Assessment (LCA) on these novel technologies for the continuous production of algal biobutanol are based on unique and until now unpublished data from a pilot plant. Applying an upscaling approach the environmental impacts of algal biobutanol production at large-scale (20 ha plant) are presented for different scenarios. The results of the prospective LCA show that the higher productivity of the genetically engineered cyanobacteria *Synechocystis PCC6803* and its specific feature of discharging the product biobutanol into the medium has a positive impact on the environment. However, electricity demand required for algae cultivation and product harvest overcompensates this advantage. The scenario calculations show that a positive climate gas balance can only be achieved if renewable energy is used.

These results indicate the importance of genetic engineering and the energy transition for a fully renewable electricity supply to take full advantage of their environmental potential. Besides, the importance of applying upscaling approaches in LCA for a fairer comparison with mature reference technologies is demonstrated.

1. Introduction

To combat climate change, we need technical and social innovations to replace fossil energy consumption with renewable energy. The focus is on the mobility sector, as the savings potential realized here is not sufficient to achieve the ambitious political goals for climate-neutral mobility by 2050. Already by this year (2020), 10% of the energy used in the transport sector of every EU country should come from renewable energy sources such as biofuels [1]. As biofuels create less greenhouse gas emissions than fossil fuels, this will help the EU to meet its long-term target of reducing greenhouse gas emissions by 85-90% (compared to 1990) by 2050 [1].

Technological innovations such as fuel production with genetically engineered microalgae and cyanobacteria had to be evaluated at the earliest possible stage of development, especially concerning their climate and environmental compatibility to inform, advise and guide decision-makers in research, politics, and industry. While first risk [2] and acceptance studies [3] have already addressed this issue, the probable environmental implications of this technology have so far not been analyzed or quantified in LCA studies in the literature [4], which underlines the novelty of the present work.

In general, fuels from microalgae are considered innovative and promising and complying with the EU sustainability criteria for biofuels and bioliquids [5,6] since they are cultivated in closed technical systems on industrial or marginal land and can be cultivated with seawater and nutrients from organic waste streams, such as from biogas plants. Therefore, microalgae and cyanobacteria have been intensively investigated according to their feedstock potential for producing biofuels [1,5]. However, algae biofuel production is not yet economically feasible and further R&D efforts are needed to increase biomass and fuel productivity and efficiency and reduce production costs.

Due to the versatility of microalgae, they can be used to produce different fuels: from TFA biodiesel [7], over biomethane or biobutanol via anaerobic fermentation [8] to hydrogen [9,10]. For large-scale energy production, large open ponds are considered to be the most appropriate approach, although this technology is not innovative and can offset some of the environmental advantages of microalgae [11–13]. This is why there are combined and interdisciplinary approaches towards innovative processes for producing third-generation algal biofuels. One of these approaches is the genetic engineering of microalgae and cyanobacteria to significantly speed up strain selection and optimization and increase their productivities. Beyond that, it is the vision of biologists and process engineers to use the ability of some microalgae to produce fuel precursors or even fuels that can pass the cell wall (such as biobutanol) and be excreted into the culture media from which it can be harvested [14–16]. In that way, the fuel precursor can be harvested from the cultivation media and the microalgae can continue growing. This way, the biofuel production remains under steady conditions, instead of “killing” the microalgae by destroying the cell wall to obtain the raw material for the production of biofuels. This so-called “milking”-process facilitates and reduces the energy demand for the ongoing recultivation of the microalgae and the harvesting step, the dewatering as well as fractionation of the algae biomass.

In this paper, we are focusing on the production of 1-butanol (referred to here as biobutanol) by the genetically engineered cyanobacteria *Synechocystis PCC6803*, which showed very high productivity at a lab and pilot scale. It can be expected that the direct release of the fuel precursor to the culture medium results in electricity saving related to harvesting a biomass fractionation. [17]. Biobutanol has many advantages over other alternatives, including its low vapor pressure, high energy density, and ability to directly replace gasoline (Bippes, M. Fuel Matrix Definition. Unpublished Work. 2017). The higher energy density of butanol compared to ethanol allows for better fuel efficiency in vehicles, giving the consumer better gas mileage [18]. Besides, the physical properties of butanol allow for direct use (fuel blend with gasoline in higher proportion than ethanol) in conventional engines without major engine modifications [19].

With a Life Cycle Assessment (LCA) approach, being a powerful tool to investigate a product according to its environmental impacts along the entire life span, we aim at giving insights into the opportunities and challenges of biobutanol production and showing the environmental bottlenecks as well as hotspots of the production process. For a fair comparison with fossil-based 1-butanol production, we developed and applied an upscaling procedure to model and assess algal fuel production with GE cyanobacteria at a large-scale. Since political framing and future developments can have significant impacts on the environmental performance of algal technologies, we conducted the LCA against the background of different scenarios.

2. Methodology

1.1 Goal and scope

The LCA methodology was chosen not only considering its relevance as a scientific assessment, but also the current significant impact of LCA in other issues, such as policy development, system, and component design, authorization and permissions as well as consensus-building [20]. Hence, the goal of this LCA is to provide a consistent understanding of the biobutanol production process related to environmental issues. This LCA model was developed together with biologists, technical and process engineers. Figure 1 shows the system boundaries and the considered process flow.

Emerging technologies have considerable potential for improvements, e.g. by applying economies of scale [21], as already successfully shown for other biofuel technologies [22]. The depicted biobutanol production process begins with the continuous cultivation process, which was upscaled considering three consecutive production volumes: Pre-inoculation (PBR 1), Inoculation (PBR 2), and Production (PBR 3). Following this, the product is filtrated and separated into two phases. Whereas, the biobutanol-rich medium undergoes a pervaporation and distillation step to be recovered. The residual algae biomass is then valorized through hydrothermal liquefaction (HTL), separation, and centrifugation processes.

Microalgae biofuel technology is still at an early stage of development compared to other biofuels [4,23]. According to the literature, a significant pending issue for algal biofuel LCA prevails on the input data and its characteristics, detail, and quality [20]. Consequently, for comprehensive data acquisition from lab and pilot scale installations, a systematic and elaborated questionnaire was developed to collect the required data and information on process design, technology, and equipment to model and calculate the LCA from well-to-tank. Intense and close personal exchange, virtual meetings and email correspondence complemented the discussion on the constructed data framework and proposed assumptions to bridge the data gaps. By this, as much information and original data for the LCA inventory as possible were gathered, analyzed, and adapted. Based on this inventory completed by data from other projects, literature, and the Ecoinvent database 3.2 [24], the environmental impact assessment (EIA) for a pilot-scale system (pre-study) was conducted. The production process was modeled with the open-source software OpenLCA 1.9.

The Ecoinvent database comprises raw material extraction as well as the assembly to pre-products, e.g. borosilicate tubes. However, transport, storage, and end-of-life options are excluded and not considered, as these processes were assumed to play only a minor role within the overall impacts. In addition to that, the delivery of inputs and raw materials to the production site, as well as machine abrasion and disposal, are defined to be outside this system’s boundaries (see Figure 1).

The functional unit is defined as the production of 1 kg of “engine-ready” biobutanol, which can be used directly as fuel for a combustion engine. To evaluate a specific process, it must refer to conventional fossil-based production pathways. Here, we selected the Ecoinvent database process named “Butanol production from hydroformylation of propylene” to compare and interpret the results of the biobutanol produced by the cyanobacteria.

For quantifying the life cycle impacts, the recommendations of the ILCD Handbook 2011 were followed [25]. This impact assessment method was developed and promoted by the European Commission and is already implemented in the OpenLCA software. There are 16 midpoint categories, classified into three types, and ranked according to their reliability. Thereof, we selected six categories, which are considered the most relevant ones for the process analyzed (Table 1).

Table 1 Selected ILCD midpoint impact categories [25]

ILCD Midpoint application			
Impact category	default LCIA method	Indicator	Classification
Climate Change	ILCD 2011: Baseline model of 100 years of IPCC	Radiative forcing as Global Warming Potential (GWP 100)	I
Particulate matter/ Respiratory inorganics	RiskPoll model (Rabl and Spadaro, 2004) and Greco et al. 2007	Intake fraction for fine praticles (kg $PM_{2.5}$-eq/kg)	
Resource depletion, mineral, fossil, and renewable (Depletion of renewable resources is included in the analysis, but none of the analyzed methods is mature for the recommendation)	CML 2002 (Guinée et al. 2002)	Scarcity	II
Eutrophication, aquatic	EUTREND model (Struijts et al., 2009b) as implemented in ReCiPE	Fraction of nutrients reaching freshwater end compartment (P) or marine end compartment (N)	
Land use	Model-based on Soil Organic Matter (SOM) (Milà i Canals et al., 2007b)	Soil Organic Matter	III
Resource depletion, water	Model for water consumption as in Swiss Ecoscarcity (Frischknecht et al., 2008)	Water use related to the local scarcity of water	

Midpoint categories classified as “I” deliver recommended and satisfactory results. Among these, we selected well-known and commonly used categories like climate change and particulate matter formation. Freshwater eutrophication and mineral resource depletion were chosen from category “II”, because of the expected impact of materials’- and fertilizers’ upstream production inputs used in a large-scale production system. Since this category is recommended but needs to be improved, the reliability of the results is not as high as those of the category “I”. The indicators considered from category “III” are water resource depletion and land use. They were selected based on the relevance of water demand for microalgae cultivation and industrial land occupation in the upscaled system [4]. However, these “III”-ranked categories are less recommended and need to be applied with caution.

1.2 Prospective process upscaling

Following the typical methodology of ex-ante prospective LCA [26], the insights are drawn from the lab and pilot-scale results were used to design and model a large-scale production plant (20 ha). Therefore, the best productivities achieved at lab scale and upscaling the process were used, to analyze whether an optimized, large-scale system has lower environmental impacts per unit (kg biobutanol) produced than the pilot scale. (Table 2).

Table 2 Assumptions for the prospective upscaling of the LCA model

	Lab	**Upscale**	**Assumptions**
Productivity (mg/l/d)	50 (weighted average of two strains)[a]	600[d]	Highest productivity achieved under lab conditions
Biobutanol concentration in PBR [g/l]	1	2.65	Measured Biobutanol concentration
Biomass/ biobutanol ratio	1:1 [own assumption]	0.35:0.65[d]	
Energy use [kWh/ kg biobutanol]			
- **Cultivation**	483[a]	23	Upscale by flow[b]
- **Separation/ Pervaporation:**	86 [17]	109	Upscale by flow[c], energy saving applied[e]
- **Heating and cooling**	18 [17]	23	Upscale by flow[c], energy saving applied[e]
- **Pumping**	n.a.	0.84	Upscale by flow[b] [27,28]
Hydrothermal liquefaction [kWh/kg biobutanol]			

Biobutanol productivities of *Synechocystis* PCC 6803 were extrapolated from lab-scale experiments by Uppsala University (Sweden) and Imperial College London (UK). Like this, we applied an optimistic year-round production of 360 days with average butanol productivity of 600 mg/l/d (Boatman, T., Zemichael, F., Wang, X., Harun, I., Vachiraroj, N., & Hellgardt, K. WP3 Activities and Outcomes. Unpublished Work. 2018). Since genetically modified cyanobacteria were used in a closed photobioreactor (PBR) system, a continuous production process was assumed to keep the risk of leakage low and prevent exposure and cross-breeding, as shown in the literature [11,12].[a] Guerra, T. (personal communication, March 28, 2017). [b] Guerra, T. (personal communication, September 19, 2018). [c] According to Lauersen and Kruse (2017). [d] Boatman, T., Zemichael, F., Wang, X., Harun, I., Vachiraroj, N., & Hellgardt, K. WP3 Activities, and Outcomes. Unpublished Work. 2018. [e] According to Liu et al. (2013)

The system boundaries of the pilot-scale are based on a PBR installation of the existing pilot plant in Lisbon. After analyzing different reactor types, the unilayer horizontal tubular (UHT) PBR was chosen as the best available cultivation technology, since this reactor proved to be more efficient in terms of productivity as well as material and energy inputs than a multilayer horizontal tubular (MHT) PBR (Guerra, T., personal communication, January 23, 2019).

To assess the environmental impacts prospectively and to improve comparability, scenarios with upscaled industrial production systems for algae-based biobutanol production on 20 ha were modeled and calculated.

2.2.1 Cultivation

Commercial large-scale cultivation of microalgae is almost entirely performed using open raceway ponds in the batch mode and to produce other products than biofuels [12]. The theoretical process design for this prospective and upscaled system is based on the concept and design of a cultivation unit at a pilot scale, with a continuous process and daily product (ethanol) harvesting. For such a case, continuous cultivation is considered to be a more efficient path for producing biofuels [29]. Based on this process, confidential information on the upscaling of the UHT-PBR system for ethanol was provided by Guerra, T. (personal communication, July 3, 2018). The land occupied by the reactor was assumed to be classified as an industrial area and used for 20 years to match the system and equipment lifespan. For a final cultivation scale, three consecutive production volumes have to be achieved: Pre-inoculation (PBR 1), Inoculation (PBR 2), and Production (PBR 3). The occupied area as well as the associated volume and number of units are listed in Table S 1 in the supplement.

Production and preparation steps providing less than 1 m^3 of culture were neglected and considered as lab work outside the system boundaries. The main materials of the production system were taken into account without assembling, forming, and construction processes. Whenever catalog data were used on electric devices like the blower, 70% of the total mass was assumed to be stainless steel only. The main materials used within the cultivation phase are listed in Table S 2. Pump work and culture bubbling were applied, too. Optimal pumps were selected using the flow rates (30 m^3/h, 83 m^3/h, 500 m^3/h) per reactor size given by Guerra, T. (personal communication, September 19, 2018) to ensure a culture speed of 0.5 m/s. No power for thermoregulation is considered since only spray water cooling is assumed to be used during the summer period (no additional pumping, tap water). The energy consumption for the 360 days of production can be depicted in Table S 3. Sensors and controlling equipment, as well as connecting pipes between the different production steps, were neglected. Values for operational materials like fertilizer, freshwater for cleaning purposes, thermoregulation, or fresh culture supply were calculated based on information provided by Guerra, T. (personal communication, February 4, 2019). Like this, we considered 107 g N/kg DM biomass as $NaNO_3$ and 15 g P/kg DM biomass as P_2O_5. According to literature, we consider that 62.5% of nitrogen and 90% of phosphate can be recycled within a hydrothermal liquefaction (HTL) process [30] significantly reducing the nutrient demand from primary sources, which are either limited (phosphate rock) or linked to energy-demanding production processes (Haber-Bosch process).

Bioenergy systems are assumed to be carbon-neutral since all carbon stored in the biomass is taken by the plants from the atmosphere. Consequently, the biomass has effectively removed carbon from the atmosphere (short-term perspective). Therefore, any thermochemical or biological conversion of biomass, which releases carbon dioxide into the atmosphere, does not contribute to any net additional greenhouse gases [31]. However, to achieve high productivities, microalgae and cyanobacteria need to be supplied with higher CO_2 concentrations than available in the atmosphere. CO_2 sources providing such higher concentrations can be supplied for example by coal-fired power or biogas plants. Nevertheless, we did not consider a technical supply of CO_2, but only CO_2 taken up during photosynthesis and assimilation by the algae. As it will be released while burning the biobutanol in a combustion engine, we did not include the process in the LCA.

As it is assumed that the system is running continuously, only one cleaning per year takes place, flushing the tubes with twice the water volume of the reactors and a solution with chlorine (7.0 kg) and thiosulfate (5.6 kg). To keep the freshwater demand low, recycling of the culture medium is assumed to reduce the water consumption, which totals to 433 m^3 including the water demand for the cooling system per year caused by evaporation. As 90% of the culture broth harvested can be recycled and fed back to the PBR system, the total freshwater volume per year can be reduced to 83 m^3. The yearly biobutanol production was considered to be about 2,000 t and 1,080 t of biomass as co-product since daily harvesting of around 30% of the culture was assumed. Differences in densities of biomass, biobutanol, and culture medium were not considered for technical configuration and processing.

2.2.2 Separation and harvesting

The recovery of biobutanol from dilute mixtures represents a bio-technical challenge in the photoautotrophic production of excreted biofuels which has not yet been addressed satisfyingly. The most suitable and cost-effective microalgal harvesting method is a constant matter of research [12]. Recently, four of the most promising butanol separation technologies (distillation, pervaporation, gas stripping, and ionic liquid extraction) were assessed concluding that, at present, it is necessary to make a compromise between energy requirement and operating costs [17]. Based on these findings we selected for our prospective upscaled LCA model the technique of pervaporation to separate the biobutanol from the cyanobacteria culture broth.

Following the cultivation process in the UHT-PBR, the product flow was separated using a polypropylene microfilter (Boatman, T., personal communication, January 7, 2019) into two phases: a biobutanol rich medium (ca. 65 Vol.%) and the biomass slurry (ca. 35 Vol. %) (Boatman, T., Zemichael, F., Wang, X., Harun, I., Vachiraroj, N., & Hellgardt, K. WP3 Activities and Outcomes. Unpublished Work. 2018). Consequently, the pervaporation system was implemented with a pervaporation temperature of 60 °C, including energy savings of 42.9 % related to energy integration (compared to a system without energy recovery) [17]. To match with the industrial scale, the data for the equipment for heating and cooling (Table S 4) and their energy demand were upscaled based to match a continuous process design [27]. For this, electricity input savings in large-scale production of 15% were considered [27]. The energy demand for the pervaporation pump was scaled up linearly without any economies of scale, due to a lack of information on flow rates.

Based on the data from the pilot scale, the biobutanol concentration was determined to be 2.65 g/l, therefore the pervaporation process in our model was built accordingly. However, the pervaporation model showed that a minimal biobutanol concentration of 10 g/l has to be achieved to reach a break-even point in terms of energy input and output [17]. Based on the productivity and concentration of biobutanol in the PBRs, a daily partial harvest has been calculated with a fixed volume of 30 % of the total culture, to maintain a stable biological system. The energy requirements for pervaporation were considered in the LCA model assuming a yearly operation of 360 days and a daily full-time 24-hour operation (Table S 5). Simultaneously to the harvesting process, fresh medium including fertilizer was added to achieve constant cultivation conditions.

Following the process simulations by Wagner, Lee-Lane, Monaghan, Sharifzadeh & Hellgardt (2019), in the LCA it is considered that the butanol-rich flow in the system is treated with a two-step pervaporation process and a final distillation column to purify the product. A process scheme can be depicted in Figure 2.

The pervaporation process is used to increase the biobutanol levels above the spontaneous butanol-water phase separation point. After this, it is possible to separate and recycle the aqueous phase back to the separation system and recover the biobutanol from the organic phase through distillation. Each pervaporation unit requires a heater, a condenser, and a vacuum pump to reduce the outlet pressure. The vacuum pumps are defined to be outside the system boundaries of this study, as no data was available. All aqueous streams are being recycled to minimize the amount of required fresh water and to recover the entire residual butanol.

2.3 Scenario development

Three different scenarios for the production of biobutanol were developed and analyzed. The specifications for the three scenarios and their major input parameters are shown in Table 3. The first scenario “Upscale” is regarded as the baseline scenario for a 20 ha system on which the other scenarios were built on. The specifications of the second and third scenarios remain the same while only the energy source was changed.

Table 3 Major input parameters for the scenarios “Upscale” and “Upscale + HTL”

Key parameters	Upscale	Upscale + HTL
Productivity [mg/L/d]	600	
Biomass/biobutanol ratio	0.35:0.65	
Electricity demand [kWh/kg biobutanol]	155	156
CO_2	Flow is not considered in the LCA due to the biogenic source	
Nitrogen fertilizer **[kg/kg biobutanol]**	0.35	0.13 (62.5% recycling)
Phosphorus fertilizer **[kg/kg biobutanol]**	0.04	0.004 (90% recycling)
By-product credit **[kWh/kg biobutanol]**	n.a.	3
Electricity mix (2012)	European mix (24.2% renewable) [32]	

HTL: Hydrothermal liquefaction

2.3.1 HTL and nutrient recovery

The scenario “Upscale + HTL” is based on the “Upscale” scenario, but supplemented by an HTL process, a further downstream step to valorize the biomass as well as to recycle nutrients (Figure 3). Pre-studies showed that biomass valorization should be included to improve the overall efficiency of the process, e.g. by recycling nutrients as well as increasing the energy output [33]. For this LCA, the HTL process has been considered as the most suitable technology to convert the residual algae biomass, since HTL, in general, is appropriate for the conversion of wet feedstocks [30]. Besides, HTL delivers a liquid energy carrier, so-called bio-crude oil, which can be upgraded and used as fuel as well.

The majority of HTL research has been performed using small-batch reactors, typically a few hundred milliliters in volume. The present LCA study comprises an upscaled HTL process operating 24 hours a day, with a daily feed of about 15,000 L which was designed according to Jones, Zhu, Anderson, Hallen & Elliot (2014) and Zhang et al. (2017) [34], complemented by experimental data provided by Wagner et al. (2019). As a result, residual algal slurries with 20 % DM content were processed and converted by a high temperature (350°C) and pressure (210 bar) reaction into four streams (Boatman, T., personal communication, January 7, 2019). A generic organic co-solvent (1,1 dimethyl cyclopentane was chosen as a reference) is also included to support the separation of the bio-oil from the other products. The solvent flow rate was set to 10 % of the total flow entering the HTL process (Boatman, T., personal communication, January 7, 2019). The process of solvent recycling itself was not considered to be within the system boundaries of this LCA study. However, a solvent recycling rate of 99,9% is applied as a credit according to Liu et al. (2013).

Figure 3 shows the HTL process as used in our model, the product yields, and the nutrient amount according to data from a case model [30]. This so-called “Aspen Design Case Model” was developed based on experimental results for *Nannochloropsis* and *Chlorella* as well from three other algae types from unpublished works that make this model applicable for fresh and saline water algae [30]. Table 4 shows elementary compositions and ash content of *Synechocystis* PCC6803 and the reference algae used in this study for HTL [35].

Table 4 Elementary composition and ash content of Synechocystis PCC6803

	***Synechocystis* PCC6803** [35]	**Aspen Design Case Model** [30]
Component	(Wt. %)	(Wt. %)
C	49.8	52
H	6.7	7.5
O	26.8	22
N	12.5	4.8
S	0.7	0.61
P	1.5	0.6
Ash	2.7	13

A list of equipment used in the HTL phase is given in Table S 6. For the full-scale model, all equipment inputs were dimensioned according to the upscaled flows that have to pass the system. After passing the HTL reactor, the solid phase is being removed from the product flow by a ceramic filter as a first step. Subsequently, a 3-phase separator is needed to isolate the other product phases [30].

According to the Aspen design case model [30], the HTL product contains 51 wt.% biocrude (dry), 43 wt.% of the aqueous phase, 4 wt.% of product gas, and 2 wt.% of solids, based on dry algae. Streams refer to the biomass flow as reported by Jones et al. (2014). Figure 3 shows the nitrogen balance in the product streams as estimated from experimental results based on Jones et al. (2014). The nitrogen and phosphorus bound in the solid and aqueous phases are internally recycled and used as a credit for the substitution of inputs [36] in the model, reducing the external nutrient demand during cultivation. The solid fraction from HTL requires a conversion step (such as acid digestion) to make the phosphorous bound bioavailable, before re-using it for algae cultivation [30]. However, any further processing of nutrients to enhance bioavailability as well as the separation of the solvent and bio-crude were considered to be outside the system boundaries. For the HTL process, the energy inputs are contemplated (Table S 7).

With every kg of biobutanol, 0.27 kg of bio-crude oil (HHV 39 MJ/kg) [37] are produced and considered in the LCA model as energy credit along with the biobutanol production. It is assumed that the biocrude oil is used to produce electricity with a conversion efficiency of 40 % [38]. The produced gas is not considered to be recycled or used as its amount is negligible (4 wt. %).

2.3.2 Renewable energy supply

The results of the pre-study LCA on data from small-scale pilot plant operation showed that the electricity demand for algae cultivation and harvest has a major impact on the LCA results because of the environmental impacts of the non-renewable sources-based electricity supply. Therefore, the standard European electricity mix has been changed to a renewable energy mix for the third scenario based on the conditions of the energy supply in Norway, where hydropower is dominating the electricity market with a share of 96.2 % [39]. This electricity mix scenario was chosen as an approximation to the time after the energy transition, whereas there is no real intention to import electricity from Norway. As the biobutanol separation also requires energy in terms of heat, the heat supply was changed to renewable sources (biogas).

3. Results And Discussion

In the following sections, the LCA results for the three scenarios are shown and discussed comprising both, issues regarding the modeling approach and specific scenario-related aspects.

3.1 Modelling aspects

The underlying study describes a theoretical system, which is mainly driven by assumptions on the biological system set up technically. As LCA considerations describe linear correlations, these assumptions have a significant influence on the results. In general, the level of uncertainty, mainly due to lacking data for technologies at low TRL, but also related to upscaling and modeling issues [20,40] is a challenge that limits the comparability of the results for an emerging technology with an approved and mature technology [41].

Biobutanol production with microalgae and cyanobacteria is a process that is still in its infancy and significant productivity improvements can be expected in particular if novel biotechnology is applied. For different cyanobacteria mutants, biobutanol productivities of 15 mg/l/d in 2012 [42] and 64 mg/l/d in 2017 (Miao, R., Xufeng, L., Lindblad, P., & Lindberg, P. Engineering isobutanol and 1-butanol synthesis pathways into the cyanobacterium *Synechocystis PCC 6803*. Unpublished Work. 2017) are reported. However, the setting is not comparable due to another technique of genetic engineering and differences in the production systems applied. In 2019, maximum biobutanol productivity with engineered cyanobacteria of 302 mg/l/d was achieved [43]. The literature review shows the huge potential of increasing productivity through engineering progress within only a short time. This trend indicates the opportunity to further reduce the environmental impacts of algae biobutanol production. In this study, we assume a prospective best-case biobutanol productivity of 600 mg/l/d, which was reported for a lab-scale system in 2018 (Boatman, T., Zemichael, F., Wang, X., Harun, I., Vachiraroj, N., & Hellgardt, K. WP3 Activities and Outcomes. Unpublished Work. 2018). To reach high productivities, the supply of nutrients must be adjusted. This is also true for the supply of CO_2, which is needed as additional CO_2-fertilization. Assuming carbon neutrality, these CO_2 inputs were neglected for this study. However, appropriate CO_2 accounting methods should be further developed for CO_2-dependent biofuel systems. Especially in the discussion of synthetic fuels (i.e. e-fuels), such a framework will be required.

3.2 Process contribution

The following paragraphs describe the LCA results of the three scenarios identifying the main hotspots and bottlenecks of biobutanol production.

3.3. Scenarios

The obtained results for all scenarios in the underlying study are displayed in the same way referring to the production of 1 kg of biobutanol. Especially electricity and operational materials, e.g. fertilizers and the embedded burdens of the materials used, contribute to the impact assessment results. Processes contributing to less than 2 % to each impact category were summarized as “others”.

3.3.1 Scenario Upscale

The baseline scenario “Upscale” comprises operational conditions and processes that were experimentally tested and verified at a small scale only but upscaled for this study to fulfill industrial requirements. Figure 4 shows that the highest share within climate change (38.9 kg CO_2 eq), freshwater eutrophication (0.015 kg P eq), and particulate matter formation (0.012 PM2.5eq) is resulting from the energy consumption (92 %, 90 %, and 74 % respectively). As expected, the land use category as well as the mineral depletion category are mainly related to the infrastructure like the reactor system itself (53% and 59%, respectively). However, the land use category, expressed as kg SOC, is a soil quality indicator, which is especially important for assessing the impacts on fertile land use (agriculture and forestry systems). As we are referring to an industrial scale, we are considering exceptionally the “occupation of industrial area” without any land transforming processes within the inventory of the infrastructure [25].

The SOC indicator provides directly relevant information for the assessment of a system's net contribution to the Global Warming Potential (GWP) through the effect on the soil carbon pool [44]. However, the soil mechanisms regarding carbon capture and release related to Land Use Change (LUC) are complicated and different LUC measures might be more adequate as this impact category was a crucial part of recent debates on the sustainability of biofuels [5,6].

Operational materials dominate the impact category of water depletion (52%). Here, freshwater inputs for cooling as well as for medium preparation are considered. Besides, the main contributors' electricity, infrastructure, and fertilizer inputs represent a major share in the overall LCA impacts.

3.3.2 Scenario Upscale + HTL

Compared to the baseline scenario “Upscale”, the LCA results for the scenario “Upscale + HTL” improved for all selected impact categories in different proportions (Figure 5). For climate change, a reduction from 38.99 kg CO_2 eq to 37.72 kg CO_2 eq can be observed. The absolute share of nutrients decreased from 4.7 kg CO_2 eq (Upscale) to 1.7 kg CO_2 eq. Since a credit along the production process for the biocrude oil produced is applied, the definite value on CO_2 eq for the electricity consumption is reduced from 35.94 kg CO_2 eq (Upscale) to 35.77 kg CO_2 eq. However, this saving is counterbalanced by the energy-intense HTL process itself. The main pattern of contributors to the impacts remains the same. With a reduced contribution of fertilizer, the impact in mineral resource depletion from resources for nutrient production decreases from 16.9 % to 4.6 % (related to nutrient production). Although more energy is needed absolutely, the savings concerning nutrients improve the overall results. Thus, the application of HTL proved to be beneficial in environmental terms.

3.3.3 Scenario Upscale + HTL + NO

In the third scenario, the electricity supply was changed to the Norwegian mix while the heat supply was changed to biogas from the waste stream in a Norwegian setting. In this scenario, the climate change impact decreased to an absolute value of 3.08 kg CO_2 eq. Besides, the impacts of infrastructure became more obvious in most impact categories and dominated the results, while the pattern of contribution changed. For most impact categories, the results improved. Nevertheless, the value of water depletion increased significantly (Figure 6). The absolute values of depleted water increased from 0.106 m^3 to 0.23 m^3 per kg of biobutanol. However, the ILCD methodology of accounting for water used in hydropower turbines is considered misleading. The characterization factor for “Water, turbine use, unspecified natural origin, NO” is 0.000535 m^3 water eq/m^3, which drives the water depletion impact category in the foreground system to about 48 %. The background system, which was not affected by the exchange of the electricity mix, remained the same in terms of absolute values. In literature, there is a wide discussion about the reliability of the methods and appropriate dedication of water flows to impact categories, e.g. how the water used in turbines is considered. [45] suggest setting turbine water to zero in the assessment, since there is no water consumption within the spatial boundaries.

3.4 Comparison of scenarios and fossil reference

The results of the comparison of the normalized results of the different scenarios with the fossil reference are shown in Figure 7. The “zero lines” refers to the reference system (1 kg of 1- butanol from hydroformylation), to which the results were normalized. The LCA results of the third scenario “Upscale + HTL + NO” indicate that the energy transition will have a significant impact on the environmental competitiveness of biobutanol produced with cyanobacteria compared to the fossil reference. This is true for climate change (Upscale + HTL + NO: 3.1 kg CO_2 eq/kg biobutanol versus fossil reference: 2.45 kg CO_2 eq./kg 1-Butanol) and freshwater eutrophication (Upscale + HTL + NO: 0.0009 kg P eq./kg biobutanol versus 0.0008 kg P eq./kg 1-Butanol), which shows a positive perspective for further optimizations of biobutanol production by cyanobacteria.

4. Conclusions

This paper investigates the environmental sustainability of the production of biofuels with an innovative process applying genetically engineered microalgae already at an early stage of technology development. LCA on technologies at rather low TRL is complex and difficult due to lacking data and information, but important for technology developers and decision-makers in politics and industry to identify hotspots and leverage points for providing insights at large scale production and on societally feasible scenarios. Based on these results, research and development targets as well as efforts can be steered more efficiently at a more advanced level.

The results of the prospective upscaled LCA with the scenario approach applied in this work show that the main hotspot of biobutanol production with cyanobacteria is the electricity demand and supply. The energy transition will help the process to become more environmentally friendly. As the system was based on theoretical estimations, no specific recommendations for process improvements can be given. Nevertheless, we consider biobutanol production with cyanobacteria as innovative technology, which may even become ecologically competitive to the fossil reference under the political conditions of a circular bioeconomy and a successful energy transition in Europe. Therefore, it seems promising to continue and strengthen R&D on microalgae production processes for the supply of sustainable biofuels as an alternative for bioelectric mobility, in particular for air mobility. Without a successful energy transition, it will be difficult to achieve sustainable production of biobutanol with microalgae relying on the state of the art technologies. Moreover, there is an urgent need to reduce the electricity demand for algae cultivation and product harvest significantly. However, the modular structure of the biobutanol production process based on single photobioreactors limits the possibilities to achieve significant improvements in environmental impacts by economies of scale only.

The LCA results indicate that integrating the HTL process to recycle the nutrients can reduce the environmental impacts, but large-scale need to be approved to ensure that there are any disturbances or disadvantages in the cultivation process. The combination of different production steps with rather low TRL is a challenge for upscaling due to the lack of knowledge and data. Therefore, this work is an important first step to give orientation knowledge on the environmental impacts of future biobutanol production with algae.

This study is the first LCA of biobutanol production through genetically modified algae carried out with real data. There are currently other works that address this issue [46], however, these assessments are based on assumptions from the literature to construct a hypothetical production scheme. The fact that these are purely theoretical studies, highlights the importance of the present work. The comparison of results is not possible, due to the different types of reactors and conditions that are being used. It is worth mentioning that one of the productivities used by Nilsson, Shabestary, Brandão & Hudson (2020) is also in the same range of the biobutanol productivity presented in this work (600 mg/L/d), which validates our data, that was considered high in comparison with previous literature.

Declarations

Acknowledgment

The authors would like to acknowledge with appreciation the PHOTOFUEL project partners for providing data on the Photofuel process chain.

Funding This work was supported by the European Union’s Horizon 2020 research and innovation program under grant agreement 640720.

Conflicts of interest The authors declare that they have no competing interests.

Availability of data and material All data generated or analysed during this study are included in this published article [and its supplementary information files].

Code availability Not applicable

5. References

[1] European Commission. New study investigates properties of bio kerosene fuel blends 2019. https://ec.europa.eu/energy/en/news/new-study-investigates-properties-bio-kerosene-fuel-blends (accessed December 5, 2019).

[2] Henley WJ, Litaker RW, Novoveská L, Duke CS, Quemada HD, Sayre RT. Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. Algal Res 2013;2:66–77.

https://doi.org/10.1016/j.algal.2012.11.001.

[3] Varela Villarreal J, Burgués C, Rösch C. Acceptability of genetically engineered algae biofuels in Europe: Opinions of experts and stakeholders. Biotechnol Biofuels 2020;13. https://doi.org/10.1186/s13068-020-01730-y.

[4] Benson D, Kerry K, Malin G. Algal biofuels: Impact significance and implications for EU multi-level governance. J Clean Prod 2014;72:4–13. https://doi.org/10.1016/j.jclepro.2014.02.060.

[5] European Commission. Commission delegated regulation (EU) 2019/807. 2019.

[6] European Parliament. Directive (EU) 2018/2001 of the European Parliament and of the Council of 11 December 2018 on the promotion of the use of energy from renewable sources. Off J Eur Union 2018;128:82–209.

[7] Moore K, Rösch C, Biondi N. Life Cycle Assessment to analyze the impacts from renewable electricity and nutrient cycling for algal biodiesel production. Algal Res 2020;(under rev.

[8] Wang Y, Ho SH, Yen HW, Nagarajan D, Ren NQ, Li S, et al. Current advances on fermentative biobutanol production using third generation feedstock. Biotechnol Adv 2017;35:1049–59. https://doi.org/10.1016/j.biotechadv.2017.06.001.

[9] Harish BS, Mekala JR, Babu Uppuluri K. Bioengineering strategies on catalysis for the effective production of renewable and sustainable energy. Renew Sustain Energy Rev 2015;51:533–47. https://doi.org/10.1016/j.rser.2015.06.030.

[10] Lehr F, Morweiser M, Rosello Sastre R, Kruse O, Posten C. Process development for hydrogen production with Chlamydomonas reinhardtii based on growth and product formation kinetics. J Biotechnol 2012;162:89—96. https://doi.org/10.1016/j.jbiotec.2012.06.002.

[11] Dutta S, Neto F, Coelho MC. Microalgae biofuels: A comparative study on techno-economic analysis & life-cycle assessment. Algal Res 2016;20:44–52. https://doi.org/10.1016/j.algal.2016.09.018.

[12] Rawat I, Ranjith Kumar R, Mutanda T, Bux F. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Appl Energy 2013;103:444–67. https://doi.org/10.1016/j.apenergy.2012.10.004.

[13] Rösch C, Roßmann M, Weickert S. Microalgae for integrated food and fuel production. GCB Bioenergy 2019;11:326–34. https://doi.org/10.1111/gcbb.12579.

[14] Parmar A, Singh NK, Pandey A, Gnansounou E, Madamwar D. Cyanobacteria and microalgae: A positive prospect for biofuels. Bioresour Technol 2011;102:10163–72. https://doi.org/10.1016/j.biortech.2011.08.030.

[15] Nozzi NE, Oliver JWK, Atsumi S. Cyanobacteria as a Platform for Biofuel Production. Front Bioeng Biotechnol 2013;1:1–6. https://doi.org/10.3389/fbioe.2013.00007.

[16] Miao R, Xufeng L, Lindberg P, Lindblad P. Report on designed strains and optimized processes for production of short chain-length alcohols reaching at least TRL4. Deliverable D2.3. Photofuel Project. http://www.photofuel.eu/uploads/files/photofuel-d2.3_butanol_biocatalyst_development.pdf. 2017.

[17] Wagner JL, Lee-Lane D, Monaghan M, Sharifzadeh M, Hellgardt K. Recovery of excreted n-butanol from genetically engineered cyanobacteria cultures: Process modelling to quantify energy and economic costs of different separation technologies. Algal Res 2019;37:92–102. https://doi.org/10.1016/j.algal.2018.11.008.

[18] Bevan E. Algae to butanol: the design, construction, and implementation of an automated sugar-to-fuel process. Chemical Engineering Undergraduate Honors Theses. 2011.

[19] Shapovalov OI, Ashkinazi LA. Biobutanol: Biofuel of second generation. Russ J Appl Chem 2008;81:2232–6. https://doi.org/10.1134/S1070427208120379.

[20] Chiaramonti D, Maniatis K, Tredici MR, Verdelho V, Yan J. Life Cycle Assessment of Algae Biofuels: Needs and challenges. Appl Energy 2015;154:1049–51. https://doi.org/10.1016/j.apenergy.2015.06.006.

[21] de Jong S, Hoefnagels R, Wetterlund E, Pettersson K, Faaij A, Junginger M. Cost optimization of biofuel production – The impact of scale, integration, transport and supply chain configurations. Appl Energy 2017;195:1055–70. https://doi.org/10.1016/j.apenergy.2017.03.109.

[22] van den Wall Bake JD, Junginger M, Faaij A, Poot T, Walter A. Explaining the experience curve: Cost reductions of Brazilian ethanol from sugarcane. Biomass and Bioenergy 2009;33:644–58. https://doi.org/10.1016/j.biombioe.2008.10.006.

[23] Bahadar A, Bilal Khan M. Progress in energy from microalgae: A review. Renew Sustain Energy Rev 2013;27:128–48. https://doi.org/10.1016/j.rser.2013.06.029.

[24] Wernet G, Bauer C, Steubing B, Reinhard J, Moreno-Ruiz E, Weidema B. The ecoinvent database version 3 (part I): overview and methodology. Int J Life Cycle Assess 2016;21:1218–30. https://doi.org/10.1007/s11367-016-1087-8.

[25] European Commission - Joint Research Centre. ILCD Handbook: Recommendations for Life Cycle Impact Assessment in the European context. 1st ed. 2011. https://doi.org/10.278/33030.

[26] Cucurachi S, Van Der Giesen C, Guinée J. Ex-ante LCA of Emerging Technologies. Procedia CIRP 2018;69:463–8. https://doi.org/10.1016/j.procir.2017.11.005.

[27] Liu X, Saydah B, Eranki P, Colosi LM, Greg Mitchell B, Rhodes J, et al. Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via

hydrothermal liquefaction. Bioresour Technol 2013;148:163–71. https://doi.org/10.1016/j.biortech.2013.08.112.

[28] Zhang J, Zhang Y, Luo Z. Hydrothermal liquefaction of chlorella pyrenoidosa in ethanol-water for bio-crude production. Energy Procedia 2014;61:1961–4. https://doi.org/10.1016/j.egypro.2014.12.052.

[29] Cooney MJ, Young G, Pate R. Bio-oil from photosynthetic microalgae: Case study. Bioresour Technol 2011;102:166–77. https://doi.org/10.1016/j.biortech.2010.06.134.

[30] Jones S, Zhu Y, Anderson D, Hallen RT, Elliott DC. Process Design and Economics for the Conversion of Algal Biomass to Hydrocarbons: Whole Algae Hydrothermal Liquefaction and Upgrading. 2014. https://doi.org/10.2172/1126336.

[31] Thornley P, Adams P. Policy Lessons: The Role of Policy Regimes in Maximising GHG Savings in Bioenergy Systems. Greenh. Gas Balanc. Bioenergy Syst., 2018, p. 245–60. https://doi.org/10.1016/B978-0-08-101036-5.00018-5.

[32] European Environment Agency. Overview of electricity production and use in Europe 2015. http://www.eea.europa.eu/data-and-maps/indicators/overview-of-the-electricity-production-1/assessment (accessed March 13, 2016).

[33] Rösch C, Ketzer F, Villacreses D. Generic LCA modelling. Deliverable D6.2. Photofuel Project. http://www.photofuel.eu/uploads/files/photofuel-d6.2_lca-modelling.pdf. 2017.

[34] Zhang B, Wu J, Deng Z, Yang C, Cui C, Ding Y. A Comparison of Energy Consumption in Hydrothermal Liquefaction and Pyrolysis of Microalgae. Trends Renew Energy 2017;3:76–85. https://doi.org/10.17737/tre.2017.3.1.0013.

[35] Kim HW, Vannela R, Zhou C, Harto C, Rittmann BE. Photoautotrophic nutrient utilization and limitation during semi-continuous growth of Synechocystis sp. PCC6803. Biotechnol Bioeng 2010;106:553–63. https://doi.org/10.1002/bit.22724.

[36] European Commission - Joint Research Centre - Institute for Environment and Sustainability. International Reference Life Cycle Data System (ILCD) Handbook: General guide for Life Cycle Assessment - Provisions and Action Steps. Eur 24378 en - 2010. Luxembourg: 2010. https://doi.org/10.2788/94987.

[37] Fessehaye Z, Boatman T, Hellgardt K. Identification of optimal strategies for waste utilisation and recycle. Deliverable D3.6. Photofuel Project. http://www.photofuel.eu/uploads/files/photofuel-d3.6_optimal_strategies_for_waste_utilisation_and_recycle.pdf. 2018.

[38] European Environment Agency. EN19 Efficiency of conventional thermal electricity production. 2004.

[39] Itten R, Frischknecht R, Stucki M, Scherrer P. Life Cycle Inventories of Electricity Mixes and Grid. Paul Scherrer Inst 2014:1–229.

[40] Huijbregts MAJ. Application of uncertainty and variability in LCA. Part I: A general framework for the analysis of uncertainty and variability in life cycle assessment. Int J Life Cycle Assess 1998;3:273–80. https://doi.org/10.1007/BF02979835.

[41] Seidel C. The application of life cycle assessment to public policy development. Int J Life Cycle Assess 2016;21:337–48. https://doi.org/10.1007/s11367-015-1024-2.

[42] Lan EI, Liao JC. ATP drives direct photosynthetic production of 1-butanol in cyanobacteria. Proc Natl Acad Sci 2012;109:6018–23. https://doi.org/10.1073/pnas.1200074109.

[43] Liu X, Miao R, Lindberg P, Lindblad P. Modular engineering for efficient photosynthetic biosynthesis of 1-butanol from CO 2 in cyanobacteria . Energy Environ Sci 2019. https://doi.org/10.1039/c9ee01214a.

[44] Brandão M, Milà i Canals L, Clift R. Soil organic carbon changes in the cultivation of energy crops: Implications for GHG balances and soil quality for use in LCA. Biomass and Bioenergy 2011;35:2323–36. https://doi.org/10.1016/j.biombioe.2009.10.019.

[45] Bakken TH, Modahl IS, Engeland K, Raadal HL, Arnøy S. The life-cycle water footprint of two hydropower projects in Norway. J Clean Prod 2016;113:241–50. https://doi.org/10.1016/j.jclepro.2015.12.036.

[46] Nilsson A, Shabestary K, Brandão M, Hudson EP. Environmental impacts and limitations of third-generation biobutanol: Life cycle assessment of n-butanol produced by genetically engineered cyanobacteria. J Ind Ecol 2020;24:205–16. https://doi.org/10.1111/jiec.12843.

Figures

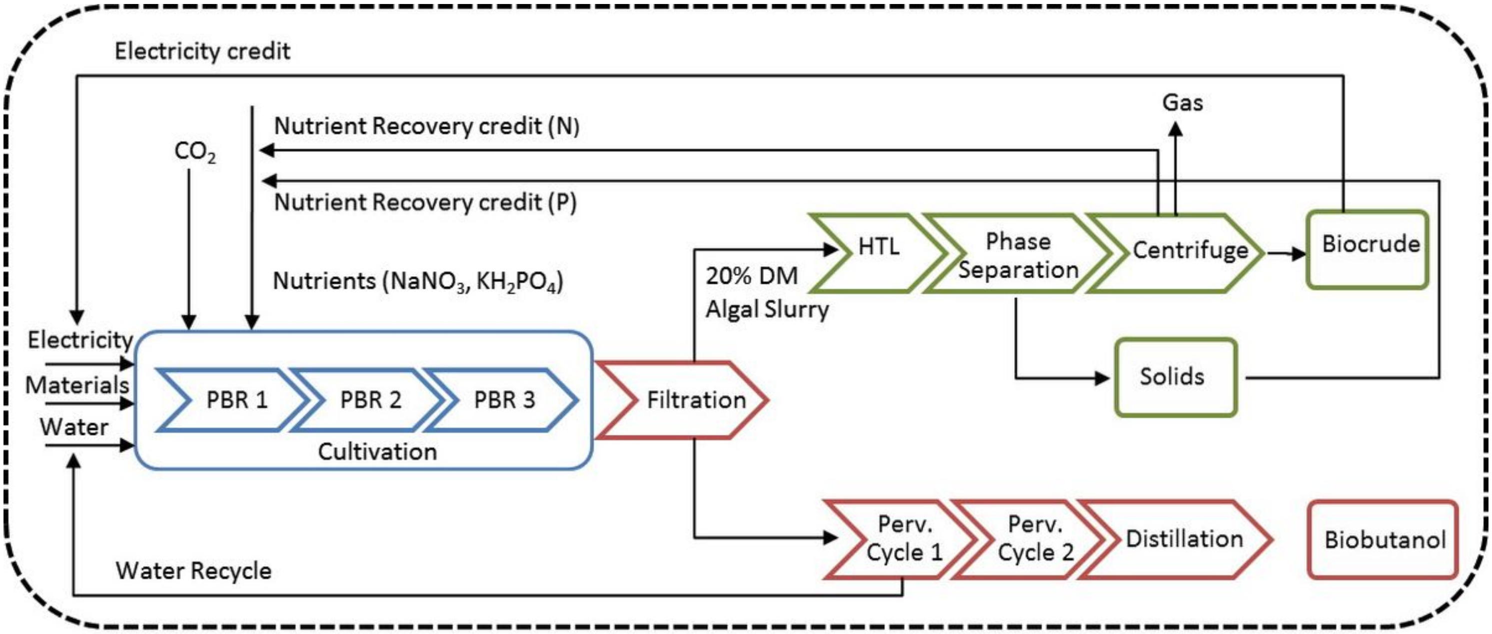

Figure 1

LCA system boundaries and process flow of biobutanol production with cyanobacteria. (PBR: Photobioreactor; HTL: Hydrothermal liquefaction; Perv.: Pervaporation; DM: Dry matter; N: Nitrogen; P: Phosphorus)

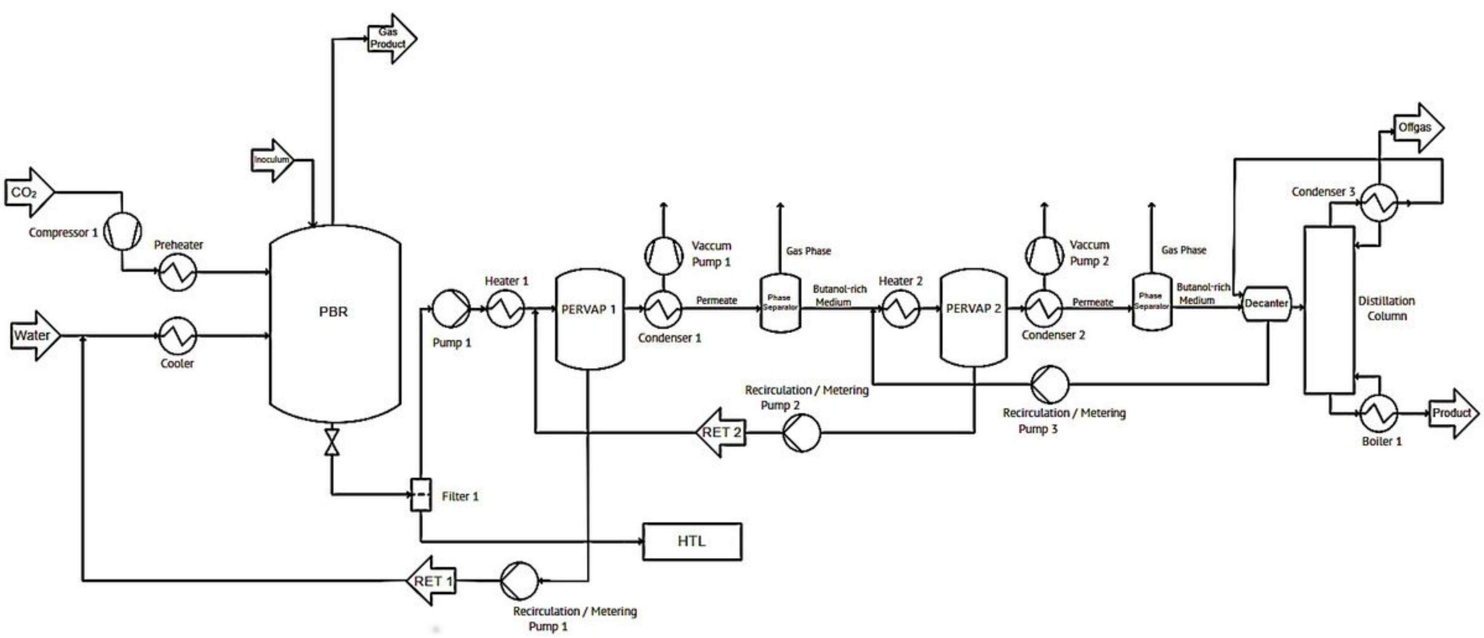

Figure 2

Flow diagram of the biobutanol production process according to Wagner et al. (2019), modified

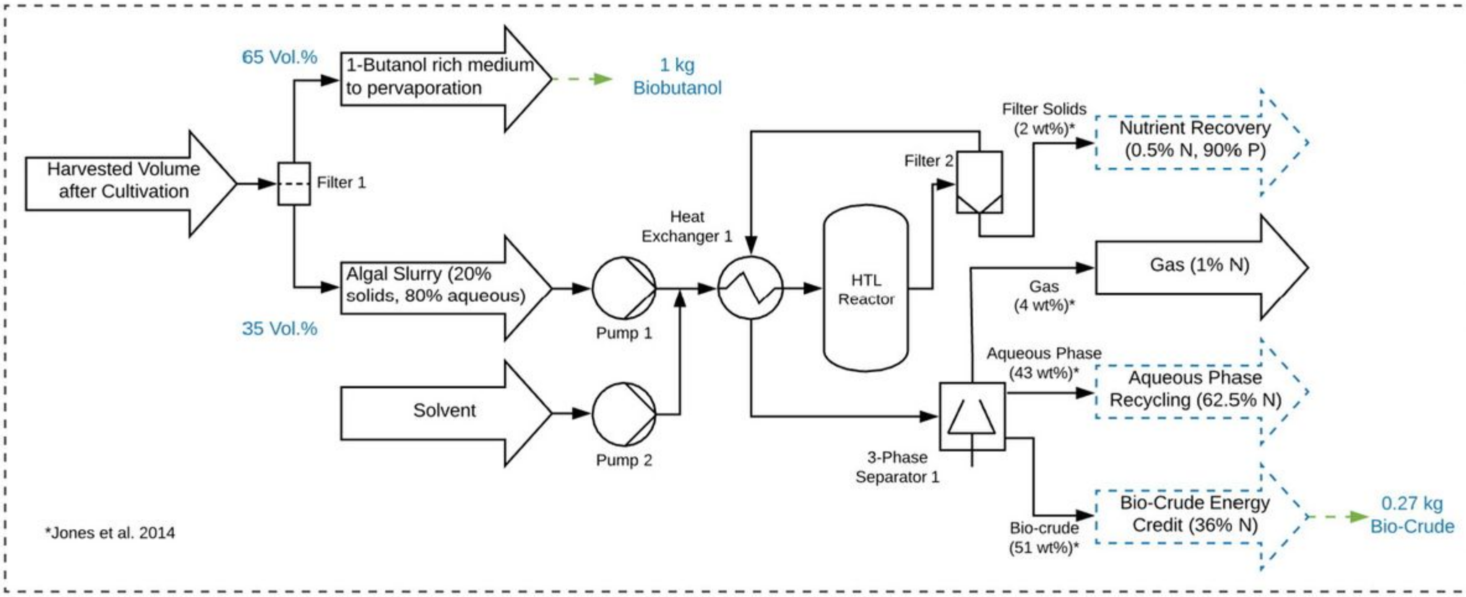

Figure 3

Flow diagram of the HTL process as implemented in the LCA model (based on [30])

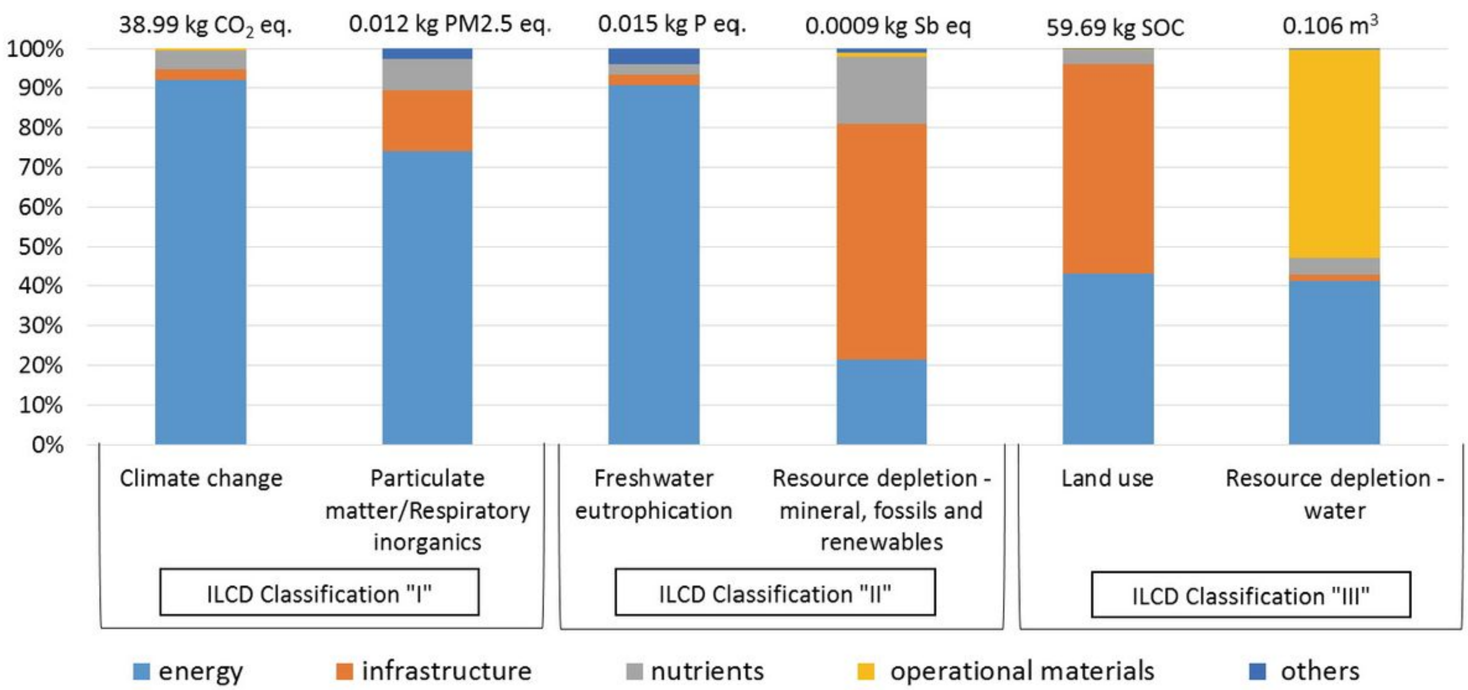

Figure 4

LCA results for biobutanol production with Synechocystis PCC6803 in UHT-PBR (Scenario Upscale)

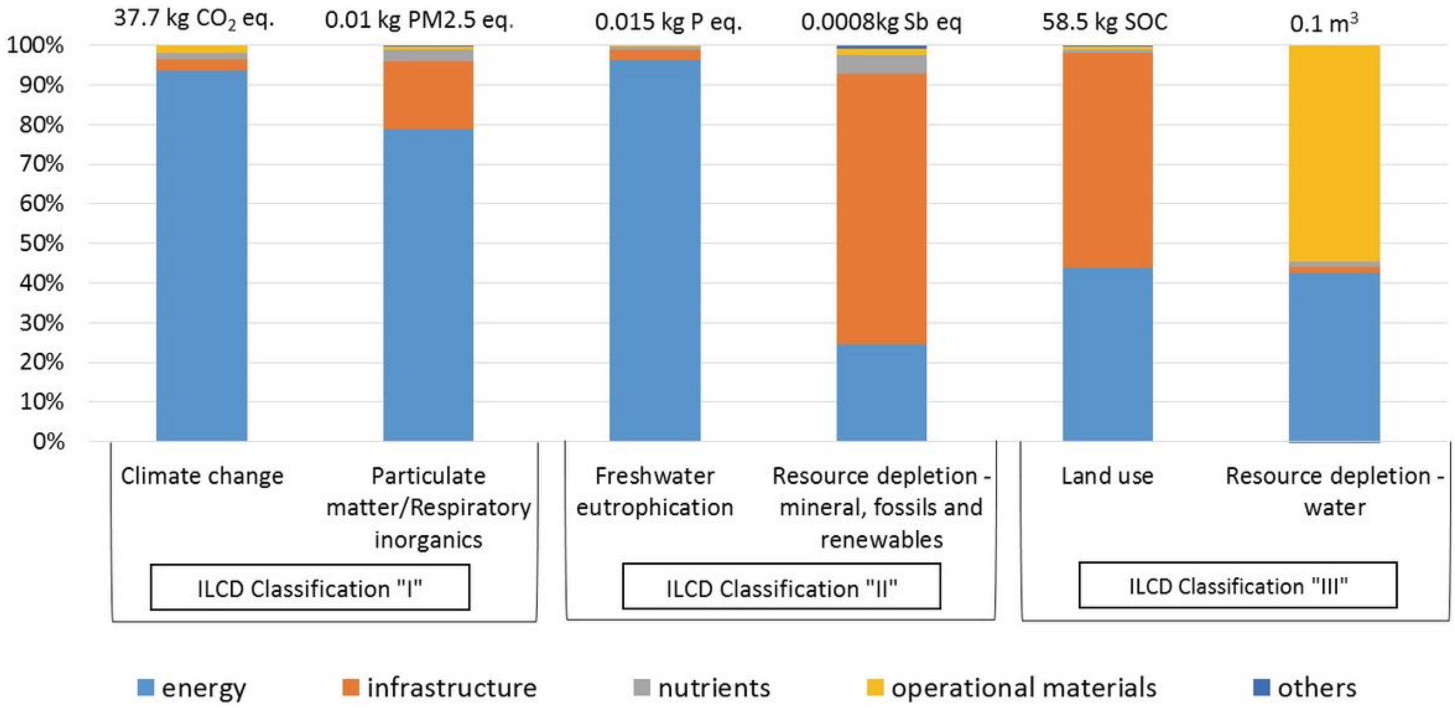

Figure 5

LCA results for biobutanol production with Synechocystis PCC6803 in UHT-PBR (Scenario Upscale + HTL)

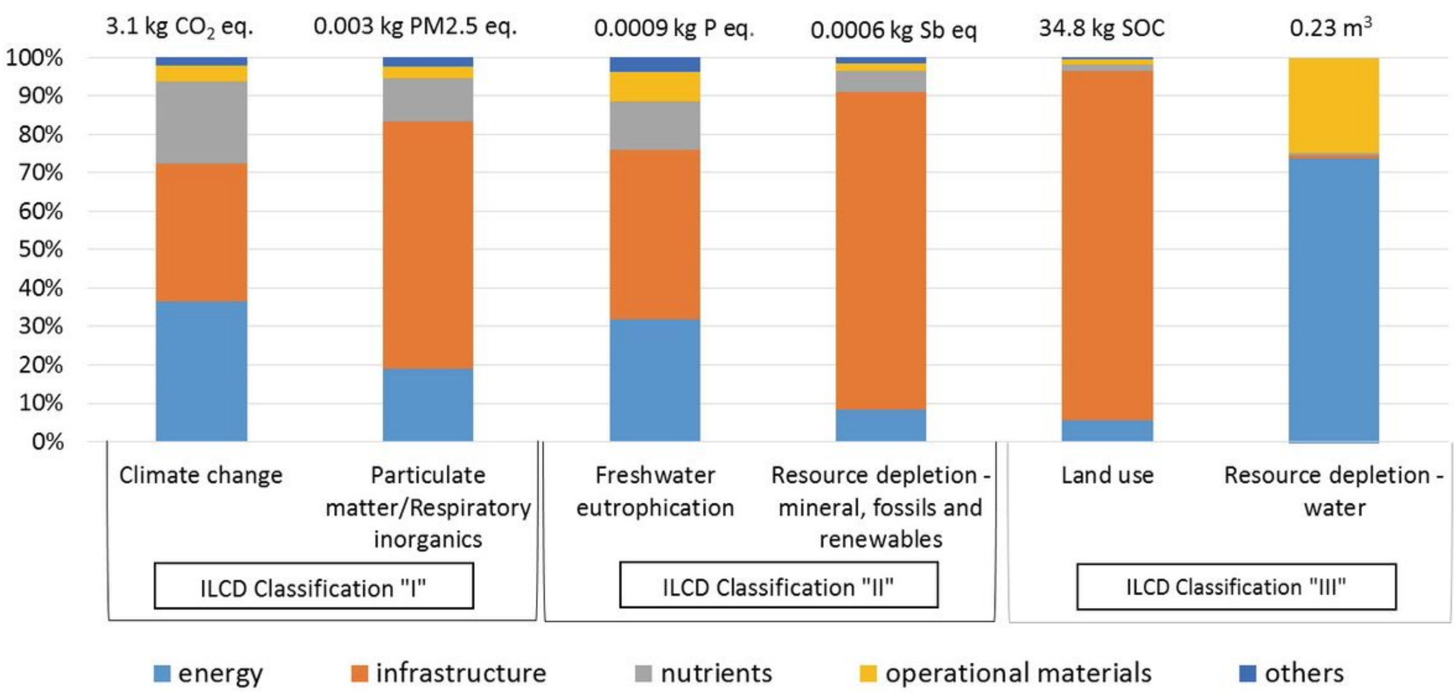

Figure 6

LCA results for biobutanol production with Synechocystis PCC6803 in UHT-PBR (Scenario Upscale + HTL + NO)

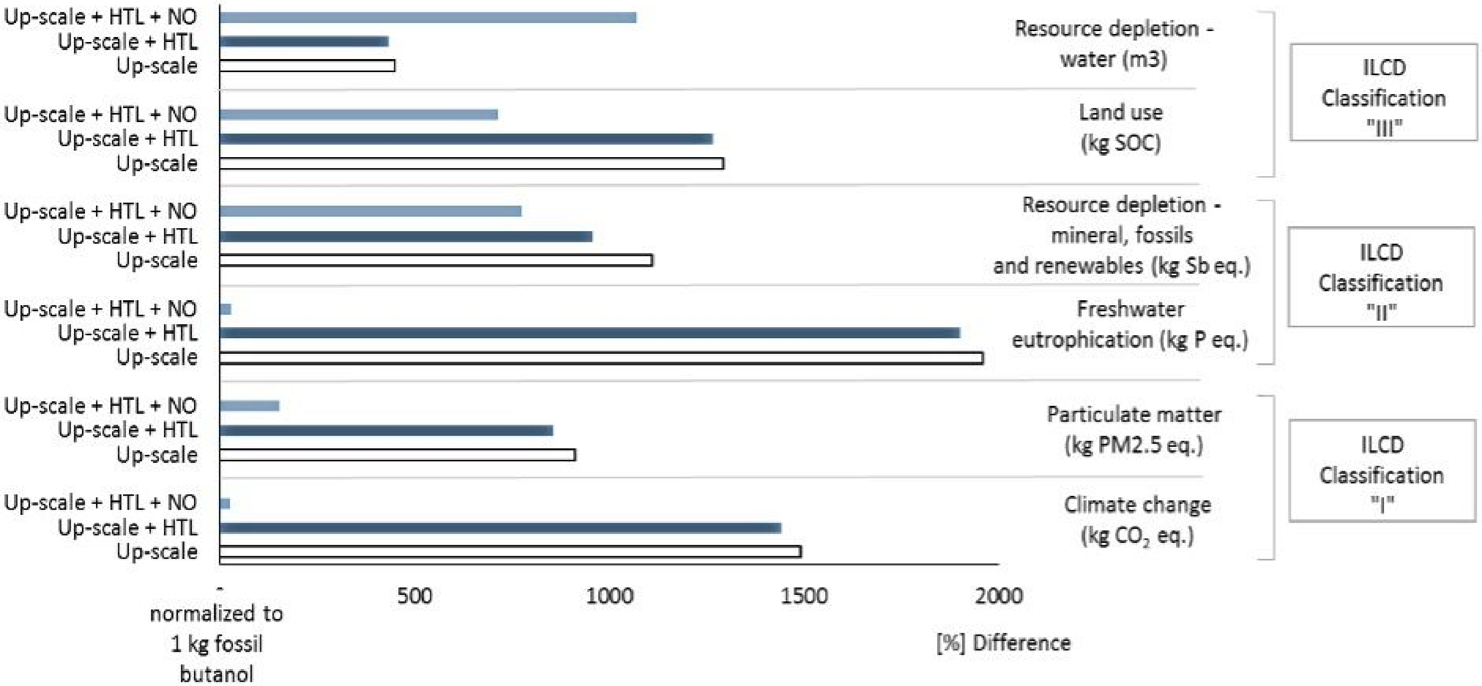

Figure 7

Comparison of absolute LCA results for biobutanol production with Synechocystis PCC6803 in UHT-PBR by scenario and fossil reference, normalized to the fossil reference system

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.docx