EU Framework Programme for Research and Innovation
H2020-Competitive Low-Carbon Energy
Call topic 11-2014

www.photofuel.eu

Photofuel - Biocatalytic solar fuels for sustainable mobility in Europe

Deliverable D1.12

Three workshops on progress and results achieved

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 640720

Editorial	
Deliverable Nº:	D1.12
Title	Three workshops on progress and results achieved
Workpackage:	WP1, WP2, WP3, WP6
Responsible beneficiary:	SYNCOM
Authors:	Jessica Varela Villarreal, Christine Rösch, Kirsten Moore, Simon Kühner, Tiago Guerra
Contributors:	KIT, UniBi, A4F
Version:	
Due date of deliverable:	30/06/2020
Version date:	12/06/2020
Contact:	jessica.villarreal@kit.edu; christine.roesch@kit.edu; tiago.guerra@algafuel.pt; s.kuehner@syn-com.com
Dissemination level:	PU-Public
Nature:	Report
Review status	WP-leader accepted 17/06/2020
	SC accepted 30/06/2020
	Coordinator submitted 01/07/2020

Table of Content

1. Introduction

The core target of the Photofuel-project is to advance the biocatalytic production of solar fuels over the entire value chain of biocatalyst development, production upscaling, fuel blending and fuel testing.

Communication and dissemination were an integral part of the proposal from the first stage proposal on and is included in three tasks:

- 1.3 Project website and general public dissemination
- 1.4 Contribution to conferences on renewable fuels, algae and biotechnology
- 1.5 Workshops on specific topics and final results

This report shows how the project results were disseminated in three workshops on biocatalyst development, biocatalytic fuel production and LCA on algae-based production systems. Accordingly, all project partners were involved in the preparation of the content, discussion of strategy and selection of event in a process which started already in the first Period.

2. Workshop on biocatalyst development

The Grant Agreement foresees holding of a workshop on biocatalyst development as a side event to the 7th International CeBiTec Research Conference on *Prospects and challenges for the development of algal biotechnology* in Bielefeld on September 24th to 27th 2017. The conference was organised by Olaf Kruse and his team, who is leader of the UniBi-group involved in Photofuel.

The motivation for proposing it as side event was to attract more participants due to the limited additional efforts compared to a stand-alone event. However, the CeBiTec-conference took place between Sunday evening and Wednesday afternoon. The conference was followed by the Photofuel plenary meeting, because many beneficiaries also attended the conference. This leaves three options:

- Have the workshop on Saturday and/or Sunday, before the start of the conference
- Workshop in parallel to one of the sessions of the conference
- Workshop between the conference and the plenary meeting on Wednesday afternoon

Relatively senior scientists dominated the audience of the conference. During the preparation of the conference it turned out that those, which were contacted to test the idea, had difficulties in attending earlier to participate in a weekend workshop and were generally not too much interested in actual lab work but more on discussion of

- working hypothesis and experimental approaches,
- cross-cutting issues between different projects or working groups,
- implications of the advancements presented on the conference for biocatalytic production

Accordingly, it was decided to discuss the outcomes of the conference with regard to the development of biocatalytic production systems in an 2h-after-session. The three Photofuel beneficiaries active in biocatalyst development in WP2 had presentations on their work in the main program of the CeBiTec Research Conference so that all attendants of the workshop started directly an intense discussion. Topics were:

- Large fragment synthetic gene assembly and gene expression in eukaryotic microalgae.
- CRISPR/Cas9(Cpf1) mediated gene knock outs.
- ptxD - phosphonate dehydrogenase from *Pseudomonas stutzeri* (*P. perfectomarina*) and its application to biocatalysts of the Photofuel project.
- Inducible promoter systems from cyanobacterial expression hosts and their importance for generating strains which can produce short chain FFA based alkanes/alkenes.
- Process design, solvent overlay capture methods and issues of scale up – the need for new bioreactor designs, outdoor cultivation of GMMs, and transfer of laboratory strains to outdoor cultivation systems.
- Progress in diatom engineering and its implications for Photofuel partners.
- The newly discovered/published eukaryotic green algal fatty acid photodecarboxylase (FAP) and its implications for hydrocarbon production from eukaryotic algae and cyanobacterial hosts.

3. Workshop on biocatalytic fuel production

To increase the critical mass and attractiveness it was decided to have the workshop on biocatalytic fuel production as side event to LIMBAC, the Lisbon Microalgae Biotechnology Advanced Course scheduled for 17^{th} to 22^{nd} November 2019 organised by A4F. LiMBAC provides an exclusive insight into advanced concepts of the microalgae industry. The course has a balanced combination of lectures and hands-on practical sessions. It covers the whole microalgae value chain, from the cell to the latest market trends. It's a unique opportunity to visit Europe's largest microalgae production site. Special attention is given to scale-up processes, large-scale cultivation, and downstream processing.The advanced course is aimed at MSc, PhD or Post-Docs in the biology, chemistry and biochemical engineering fields with some previous background on microalgae biotechnology. The course is also intended to new and established industry professionals seeking to deepen their knowledge and network with other stakeholders in the field. The course was held at the Algatec Eco Business Park, Europe's largest microalgae production site, with Green-Wall-Panels, tubular PBRs, Cascade raceways, a wide range of fully functioning state-of-the-art production units. Practical sessions used the labs of A4F, the Experimental Unit and Algatec.

Picture 1: Algatec Eco Business Park, Europe's largest microalgae production site in Lisbon

The Photofuel workshop was embedded as ‘Real Challenge’-outtake, distributed over two days as shown in the schedule below. On the first day the participants were informed on the idea behind Photofuel and what challenges occurred. Three groups were formed on the topics Biology and GMO; Biorefinery and extraction; Scale-up and Contaminant control, supported by Tiago Guerra, Simon Kühner and Luis Costa. The groups discussed about the challenges in the spare time and proposed some solutions two days later.

Theory | Practical Session | Market & Regulation | Logistics | **Real challenge**

Time	Sun 17	Mon 18	Tue 19	Wed 20	Thu 21	Fri 22
8:30 AM		Meet at hotel				
9:00 AM		Arrival at Campus				
9:30 AM		Welcome and registration	Lab 1 / Pilot Plant 1	Visit to Algatec's Industrial Site	Lab 1 / Pilot Plant 1	Engineering microalgae for biotechnology
10:00 AM		Introduction and Overview				
10:30 AM		Flash Presentations				Indust. heterotrophic microalgae
11:00 AM						Biofertilizers
11:30 AM		Coffee Break				
12:00 PM		From Cells to tons	Lab 2 / Pilot Plant 2	Industrial Archaeology	Lab 2 / Pilot Plant 2	Microalgae & biotech
12:30 PM		Light impact on PBR design		Microalgae for food		Legislation and trends
1:00 PM		Lunch				Microalgae-based lunch
1:30 PM						
2:00 PM		Design of microalgae units	Lab 3 / Pilot Plant 3	Harvesting	Lab 3 / Pilot Plant 3	
2:30 PM		Biorefinery & Extraction		Contaminants and crop protection		
3:00 PM		Aquaculture		Industrial Spirulina		
3:30 PM		Coffee Break				
4:00 PM		**Real challenge: Brainstorming**	Lab 4 / Pilot Plant 4	**Real challenge: Solutions**	Lab 4 / Pilot Plant 4	
4:30 PM						
5:00 PM						
5:30 PM		Return to hotel				
Evening	Welcome Dinner				Social Event & Conference Dinner	

Table 1: Algatec Eco Business Park, Europe's largest microalgae production site in Lisbon

Some interesting proposals came up:

- Robust chassis strains: Transfer the developed production pathways to more robust strains, which are larger (less sensitive to grazing) or can tolerate more extremophile conditions, eliminating competing or product-consuming strains from the production system
- Nutrient exclusion: Implement utilisation of special compounds like PtxD/phosphite for N-source or other essential elements
- Hostile features: Implement production of compounds with bacteriostatic or algae-static activity/self-produced antibiotics
- Dedicated separation systems: Develop a separation system dedicated for low butanol concentration
- Alternative products: Change the separation behaviour of the product by biochemical intracellular derivatisation to more volatile or more lipophilic compounds similar to octyl acetate.
- Reduce scale-up times
  - Less steps than currently --> Grow at higher volumes at lab scale; use higher scale-up ratios; have 2-step PBRs that are inoculated partially and in the subsequent step, simply open 2 valves to expand culture within the same pre-sterilized system.
  - Grow lab inoculum heterotrophically to accelerate these steps;
- Identify contaminants

  - Sample all scale-up steps and attempt isolation of present contaminants through classic microbiology techniques + identification through molecular biology; use butanol in the growth medium to increase selectivity towards contaminants consuming butanol;
- Cleaner PBR systems
  - Combine several methods of cleaning and disinfection to make it more efficient (several chemical steps, such as physical + UV + …; physical removal of biofilms and mineral deposits);
  - Disposable PBRs to use only once, but coupled with the development of the recycling process for the material of the PBR during which sterilization is achieved
- Ecosystem characterization and control
  - Focus on bacteria and fungi which can consume butanol; avoid antibiotics à abiotic stress factors, such as pH shift, nitrogen source shift…
  - Directed evolution towards production strains which are more salt resistant of low pH tolerant
  - Allelopathy: engineer the butanol production in a different organism with allelopathy characteristics to prevent other organisms from contaminating the reactor;
- Alternative products/strains
  - From non-GMO *Synechocystis*, possible to obtain interesting bioproducts, photoautotrophically, such as acetic acid (base chemical) or polyhydroxybutyrate (PHB, which is a biopolymer)
- GMO solutions
  - Introduce yet more genetic modifications into the organism which make it tolerant to high pH, high salinity, low nutrient concentrations or long periods of nutrient scarcity (increase accumulation/luxury consumption of a nutrient), conditions to which contaminants would not survive.

Picture 2: Discussion of the workshop results

4. The LCA Workshop

A workshop addressed to LCA algae experts on the topic "**LCA on upscaled algae production systems: challenges of data gaps and modeling"** was organized by KIT and took place in Frankfurt, the 24th of September 2019, from 10:00 am to 03:30 pm at the Holiday Inn Frankfurt Airport Hotel. The workshop had the following agenda.

09:45 – 10:00	Registration
10:00 – 10:15	*Welcome* *Christine Rösch, opening and welcome*
	<u>Topic 1</u>: Challenges of data gaps and uncertainties in modeling
10:15 – 10:45	Tackling data gaps of LCA in upscaled production system modeling - Data collection and development of proxies *Norbert Kohlheb;* Environmental and Biotechnology Centre (UBZ-UFZ) Leipzig, Germany
10:45 – 11:15	Challenges of upscaling innovative processes in LCA - Case study of butanol production by *Synechocystis PCC 6803* *Kirsten Moore;* Karlsruhe Institute of Technology (KIT-ITAS), Germany
11: 15 – 12:00	Discussion
12:00 – 13:00	Lunch
	<u>Topic 2:</u> Experiences with upscaled systems and projects
13:00 – 13:25	Design and assessment of algae production systems at Wageningen University (Netherlands) *Ellen Slegers,* Department of Social Sciences –Wageningen University, Netherlands
13:25 – 13:50	LCA of microalgae: too much optimism? Can we escape our fate? *Olivier Bernard*; INRIA, France
13:50 – 14:20	Coffee break
14:20 – 14:50	Hot spots in biodiesel production from microalgae and land oil-seeds *Michele Aresta;* IC^{2}R srl and CIRCC, Bari, Italy
14:50 – 15:30	Discussion and Conclusions
15:30	Closure of the Workshop

Objectives of the LCA Workshop

The objective of the workshop was the presentation and discussion of methodological approaches to perform high quality LCA on microalgae processes and products. Since algae cultivation and the linked upstream and downstream technologies are still under development and are characterized by a low TRL level, only data from lab and pilot scale experiments are available for conducting a LCA. Dealing with the uncertainties of modelling an upscaled microalgae production system and deriving data for such large-scale systems is a great challenge and a crucial task, as assumptions made in this process will have a significant impact on the environmental impacts of algal biomass and biofuel production.

Summary of the results of the LCA workshop

The workshop revealed the importance of LCA for environmental assessment of new technologies at an early stage of development in order to know how to improve the technology from the biological and technical point of view. The main challenges and lessons learned were addressed and discussed in the workshop.

The main challenges related to data collections and data gaps were:

(1) LCA experts need to build up a confidential and trusting working relationship with technology developers and pilot scale plant operators. This process can take some time, but is crucial to convince people to share their data. It is recommended that experts visit the production plant for face-to-face meetings with engineers to collect data as specific as possible. At the engineers and operators', efforts for preparation of LCA input data are often underestimated, not embedded in the workplan and under-budgeted.

(2) Frequently, LCA experts have to deal with data gaps after the collection of experimental data. A decision tree for dealing with data gaps is proposed (see Figure 1). Empirical data is preferably used, but data from literature is often applied to close the data gaps.

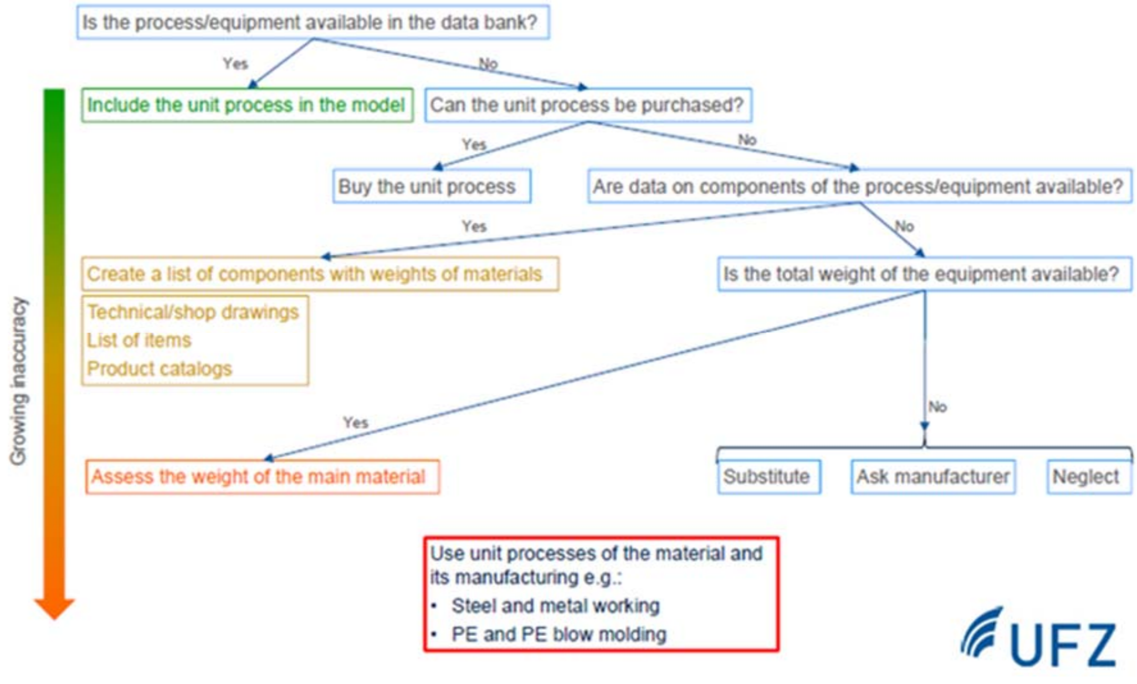

Figure 1: Decision tree for dealing with LCA-data gaps (Kohlheb *et.al.* 2019[1])

(3) Engineers and technology developers are not always aware of the requirement to provide sophisticated, reflective, detailed, and accurate data for the LCA. Thus data as given is often not suitable (i.e. equipment in place was used as-is and not optimized at all) to be applied as such in the LCA. Many given data are only applicable to prepare proxies. LCA experts need to complement real data from pilot plants with inventory data (e.g. Gabi or Ecoinvent that is not always up-to-date), modeling results from plant design and engineering and literature data. If the database is not sufficient, the calculation of an unreliable full scale ISO LCA should be avoided in favour of indicative process evaluations on base of the available information (e.g. input substrates, chemicals, products and residues).

The main challenges related to upscaling and definition of large-scale systems based on pilot-scale systems were:

(1) Integration of the competences of engineers (materials, mechanical, electric), phycologists, and chemists is essential to make a correct perspective evaluation of the potential of microalgae. An interdisciplinary team working on LCA needs to agree on the objectives and assumptions for the upscaling process. Besides, they need to develop a common understanding and vocabulary in order to work together smoothly, to learn from another and to find solutions on how to do upscaling. Teamwork and brainstorming are very important. In order to use every possible product it is essential to explore new areas of the product and to explore new areas of their process and technology.
(2) The definition of proxies in the development of the LCA model is challenging respectively if there are differences between data provided by technology developers and available unit processes of LCA data banks to develop the model. If there are no similar process units in the LCA information and data collection, LCA experts have to model their own process preferably with the support of process engineers. It has to be noted that perspective LCAs can be done only with a clear view of technology innovation: it does not make sense to consider today technologies for tomorrow production.
(3) Linear upscaling with the same process design, materials, equipment, and material and energy flows as it was used in the pilot plants may only be a bottom-line proxy in modular process design.
(4) However, for upscaling, economies of scale have to be considered and changes in physical conditions of bigger geometries (i.e. mixing), different materials for construction and different equipment (i.e. pumps, mixers, and cooling-heating) are required. Even the design of the plant may differ in order to profit of scale-of-unit-effects for improved economic-, technical- and environmental performances. Matching of technologies with different maturities from low to high TRL levels, to model the complete process from inoculum production until refining of the algae product, is tricky. For non-linear upscaling, there is engineering support available by the applying software such as SuperProDesigners or Aspen.
(5) Responsibility of LCA experts is rather high as they are regarded as environmental "judges" on new technologies by measuring and evaluating the perspectives of replacing

[1] Norbert Kohlheb, Andreas Aurich, Steffi Hunger, Roland A. Müller. 2019. Tackling data gaps of LCA in up-scaled production system modeling. *LCA on upscaled algae production systems: challenges of data gaps and modeling*, Workshop. Frankfurt, 24th of September.

an established technology or process with a new one. However, researchers conducting LCA are experts in their field and do not always have sufficient process engineering knowledge required for upscaling and for the design of large-scale plants. Besides, there is no agreed procedure for upscaling approaches, the increase of robustness of assumptions, or the application of learning curves. Moreover, no common understanding how to deal with the high level of uncertainty associated with upscaling in LCA exists, except to provide upmost transparency of modelling and assumptions made.

(6) If results are not as good as hoped for, LCA experts sometimes have to deal with a critical view on the LCA results, because nobody ever wants to hear “bad” results. In such cases the impact of the most important process steps or input substances should be explained and options for process improvement given. This might support the further development of the process and eventually lead to the expected superior performance, companies require for marketing of the new and more sustainable technology.

(7) LCA experts need to be courageous and independent to address crucial scientific questions from a socio-technical perspective, such as if the technology or process assessed is environmentally feasible, e.g. will produce renewable energy without the emission of additional greenhouse gas emissions. Having a closer look to an emerging technology such as algal technology LCA experts can turn a crystal ball into a pink crystal ball if mainly positive assumptions (e.g. estimated productivities are two times higher than state-of-the-art in pilot plants) are applied. Such a positive view of a technology is justifiable as an instrument to identify under which hypotheses the environmental impacts are more suitable or even better than the reference. If even under optimistic frame conditions the LCA is not as promising as expected in the beginning, LCA experts have to indicate whether this is a promising development or major changes such as diverting into a completely different technology or process should be made.

(8) For upscaling already at early staged model-based LCA, it is important to consider all relevant indicators, such as growth rates of strains, optimal temperature, optimal light exposure (indoor-outdoor), geometry of bioreactors, and geographical location of reactor systems; and also unfavorable parameters, as instabilities in production (i.e. cultural collapses, biofilm formation, effects of outdoor conditions). Electricity consumption for the cultivation of algae, pumping, cooling and heating need to be upscaled carefully as it is always a major hotspot found in the different categories of LCA.

(9) Since climatic conditions (not only temperature and irradiation, but also air and water quality) and the definition of system boundaries have significant influence on the LCA results of upscaled processes, LCA experts have to carefully define the relevant parameters, such as the source of energy and management of co-products and emissions. In addition, the kind of land (marginal, polluted or industrial land), the source of fertilizer and water source (e.g. residues from fishes and biogas plants) and the supply of CO_2 (e.g. from a biogenic source such as biogas or fossil-based or industrial supply from chemical plants) must also be carefully defined. Moreover, assuming the use of renewable energy in the upscaled LCA, the competitiveness of algal biomass (oil, proteins, polyols) may increase by reducing or even avoiding the demand of non-renewable energy with high greenhouse gas emissions.