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Scientific Life article: Trends in Biotechnology

Engineering biocatalytic solar fuel production: the PHOTOFUEL consortium

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Abbreviations

CI compression ignition, **FAMES** fatty acid methyl esters, **HVO** hydrotreated vegetable oil, **LCA** lifecycle assessment, **SI** spark ignition, **TEA** techno-economic analysis,

Keywords (2-6)

Fuels, photo-biomanufacturing, engine tests, LCA, TEA

Abstract

The EU Horizon2020 consortium PHOTOFUEL joined academic and industrial partners from biology, chemistry, engineering, engine design, and lifecycle assessment, making tremendous progress towards engine-ready fuels from CO₂ via photosynthetic microbes. Technical, environmental, economic and societal opportunities and challenges of feasible production at scale were elucidated to guide future technology realization.

Introduction

PHOTOFUEL was an interdisciplinary collaborative effort to engineer and assess the capability of producing liquid fuels directly secreted from engineered photosynthetic microbes using sunlight and CO₂. The consortium was formed with 13 interdisciplinary industrial and academic partners across the entire sustainable fuels value chain: biocatalyst development and cultivation, process engineering, fuel research, and engine design, complemented by environmental, economic and social impact assessment. From gene to tank to economics, holistic, systematic and interdisciplinary investigations gave first insights into the real challenges facing light-driven bio-fuel-processes. Several break-through findings and insights arose during this collaborative effort, including demonstration of novel biological routes to fuels, advances in photo-microbial cultivation and fuel separation, techno-economic barriers, environmental benefits and public acceptability. To realize sustainable, commercial fuel photo-biomanufacturing, stable high-yield cultivation and fuel extraction at scale under non-sterile conditions using nutrient recycling, reliable CO₂ supply and renewable energies are crucial.

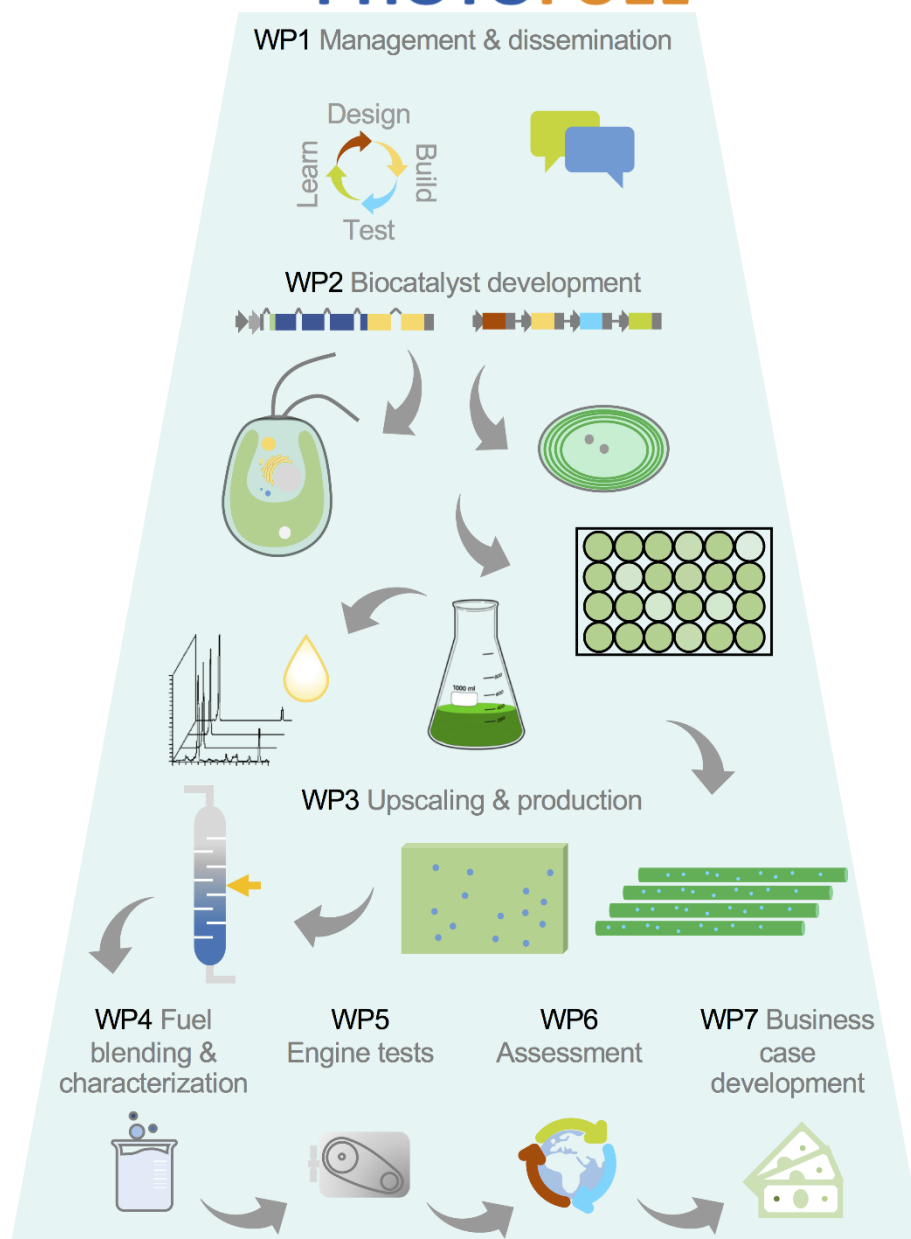
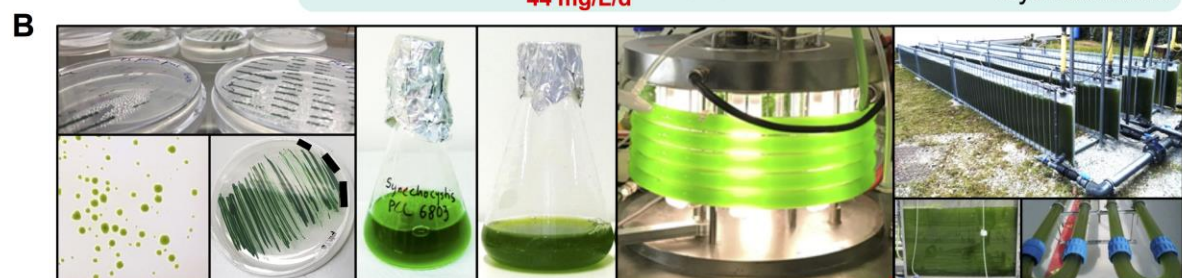
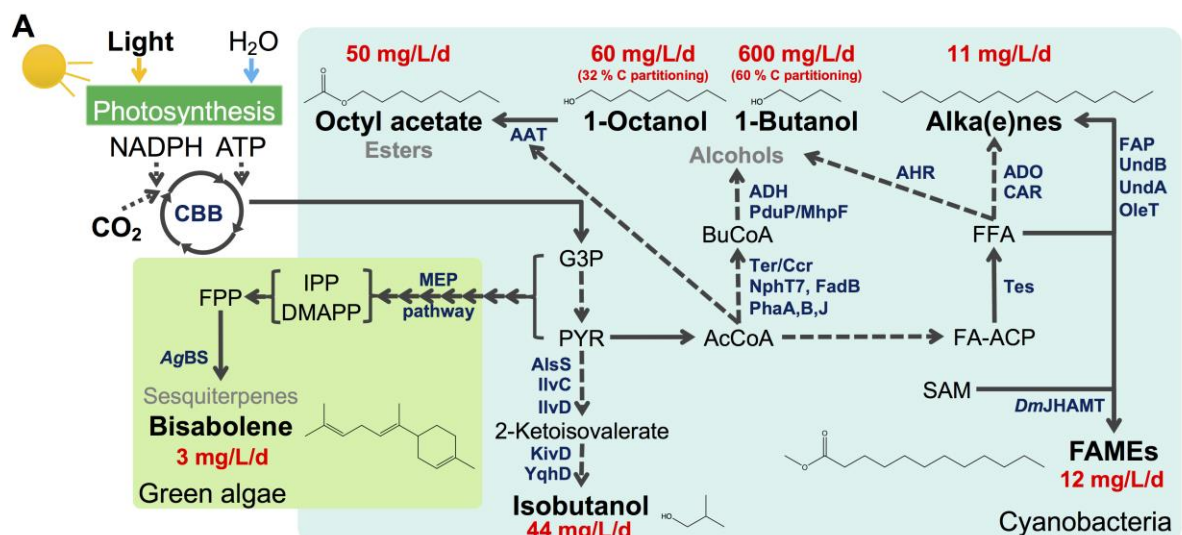


Figure 1: PHOTOFUEL project overview with work packages (WPs). WP1: Project management and dissemination accompanied by regular meetings and conferences. WP2: Optimized genetic parts were transferred to cyanobacteria (*Synechocystis* PCC 6803 and *Synechococcus* PCC 7002) and *Chlamydomonas reinhardtii* (eukaryote) to engineer fuel generating pathways. Fuel production at lab-scale was improved using design-build-test-learn cycles to optimize carbon flux to fuels. WP3: Engineered and wildtype strains were cultivated in photobioreactors up to 1400 L and downstream processing techniques compared. WP4/WP5: Promising drop-in fuel candidates were blended with conventional fuels, characterized, and engine tested. WP6/WP7: Social, economic, and environmental impacts of the whole lifecycle and business cases were also analyzed.

Challenges of large-scale photo-biocatalysis

We engineered cyanobacterial hosts to produce liquid fuels from CO₂ with great success: isobutanol [1–3], 1-butanol [4], 1-octanol [5], octyl acetate [6], and fatty acid

75 methyl esters (FAMES) [7] (Fig. 2). For some, the most efficient channeling of fixed
76 carbon to heterologous fuel product reported to date was achieved (>60 % for 1-
77 butanol, >30 % for octyl acetate). Alkane, alkene [8], and sesquiterpene [9] pathways
78 were also implemented in a green microalga. Different physical behaviors were
79 observed for each fuel product: hydrophilic molecules (<C₆) accumulated in the culture
80 broth [3,4,10] while hydrophobic products (>C₈) were collected in solvent overlays [5–
81 7,9]. We found that the majority of flux towards engineered metabolites occurred
82 concomitant with cell growth, rather than stationary photosynthetic biomass acting as
83 a solar biocatalyst. Issues such as transgene instability [5], metabolic burden [5],
84 product toxicity [8], pathway competition for, and low abundance of, precursors [4,9],
85 low enzymatic activity and intermediate channeling [7,9], and inefficient product export
86 could be overcome in these investigations. Gene expression inefficiencies, especially
87 for multi-gene pathways, were partially resolved by different gene loading strategies
88 [3,9] and combinations of regulators [4,7]. Engineering cyanobacteria was
89 straightforward given homologous recombination (HR) capacities, suitable plasmids,
90 and inducible promoter systems. Eukaryotic microalgae were generally easier to
91 cultivate, yet require advances in robust, tunable gene expression and multi-gene
92 pathways to match cyanobacterial tractability. Novel advances in metabolic
93 engineering were achieved in all of these organisms, however, practical issues and
94 hurdles to bioprocess scale-up became rapidly apparent. For example, we
95 experienced conflict between engineering of domesticated laboratory strains and
96 scale-up infrastructure optimized for more robust strains which lack genetic tools.
97 Nevertheless, we found that many domesticated and engineered hosts were
98 amenable to some of the stresses of scaled cultivation and that adaptations could be
99 applied to handle pumping shear, environmental fluctuations, and contaminants.



C

Challenges	Measures
High enzyme performance	Enzyme selection, engineering & overexpression
Precursor availability	Knock-down / knock-out of competing pathways
	Upregulate upstream reactions & bypass inefficient reactions
Balanced & sufficient flux	Balance expression & cofactors
Tools for efficient multi-gene expression	Tool development for robust strains
Fast & high cell-density growth	Media optimization, strain choice, light harvesting & C fixation efficiency
Growth vs. production dilemma	Systems approaches: Growth-coupling, 2-stage process
Genetic stability	Advance fundamental understanding
Low metabolic burden	Modular, controlled expression of pathways
	Systems-level optimization: dynamic simulation / design algorithms
Product tolerance (no toxicity)	Choice of product & capture: in-line 2-phase extraction
Efficient export	Product capture: in-line 2-phase extraction
Consumption by contaminants	Product capture: in-line 2-phase extraction
	Choice of product (indigestible molecules)
	Reactor ecosystem control (synthetic consortia, co-culture)
	Novel nutrient supply systems / extreme reactor conditions
	Reduced volume of gas exchange (mixing by pumping)
Product evaporation	Plant design (e.g. immobilization of bio-catalyst)
Biocatalyst robustness: shear stress, environmental fluctuations, extreme conditions, grazers, outgrowing contaminants	Environmental condition control (heating / cooling, artificial lighting) vs. choice of strain
	Advance knowledge on influence of variable outdoor conditions on production
	Reactor ecosystem control (synthetic consortia, co-culture)
	Novel nutrient supply systems / extreme reactor conditions
Low operating costs & energy demand	Simple DSP, high-titer production, low cost reactors, worker qualification / automation, reduced cooling & heating (adapted strains), local resources, waste valorization
High GHG saving potential	Nutrient recycling
	Biogenic CO ₂ supply
	Use of renewable energy
	Waste re-use
Low-energy separation	Increase cell densities & C partitioning to product (= volumetric productivity)
	Select <i>in situ</i> extractable or skimmable products
Public acceptance	Keep environmental promises; affordable costs

Figure 2: A Overview of implemented synthetic metabolic pathways and achieved productivities at lab scale. Most products are derived from central carbon metabolism, but require the implementation of several enzymatic steps and further modifications (e.g. knock-outs). Enzyme and chemical abbreviations: **IPP** isopentenyl pyrophosphate, **DMAPP** dimethylallyl pyrophosphate, **FPP** farnesyl pyrophosphate, **G3P** glyceraldehyde 3-phosphate, **PYR** pyruvate, **AcCoA** acetyl-CoA, **BuCoA** butyryl-CoA, **FA-ACP** fatty acyl-acyl carrier protein, **FFA** free fatty acid, **SAM** S-adenosyl methionine, **FAMES** fatty acid methyl esters, **CBB** Calvin-Benson-Bassham cycle, **MEP** methylerythritol phosphate, **AgBS** *Abies grandis* bisabolene synthase, **AAT** alcohol acetyltransferase, **AlsS** acetolactate synthase, **IlvC** ketol-acid reductoisomerase, **IlvD** dihydroxy-acid dehydratase, **KivD** alpha-ketoisovalerate decarboxylase, **YqhD** *E. coli* alcohol dehydrogenase, **PhaA** acetyl-CoA acetyltransferase, **PhaB**

acetoacetyl-CoA reductase, **PhaJ** enoyl-CoA hydratase, **NphT7** acetoacetyl-CoA synthase, **FadB** 3-hydroxybutyryl-CoA dehydrogenase, **Ter** *trans*-enoyl-CoA reductase, **Ccr** crotonyl-CoA reductase, **PduP** aldehyde dehydrogenase, **MhpF** acetaldehyde dehydrogenase, **ADH** alcohol dehydrogenase, **AHR** aldehyde reductase, **Tes** thioesterase, **CAR** carboxylic acid reductase, **ADO** aldehyde deformylating oxygenase, **DmJHAMT** juvenile hormone acid O-methyl transferase, **OleT** P450 fatty acid decarboxylase from *Jeotgalicoccus* sp. ATCC 8456, **UndA** nonheme iron oxidase responsible for 1-undecene generation in *Pseudomonas aeruginosa*, **UndB** desaturase-like enzyme converting medium-chain fatty acids into 1-alkenes, **FAP** fatty acid photo-decarboxylase. **B** From plate level to larger photobioreactors, production dynamics for most of these products were investigated. **C** Overview of challenges, requirements, and measures to integrate strain engineering with industrial photo-bio-processes. Abbreviations: **DSP** downstream processing.

Culture instability presents the biggest limitation to mass production

Fuel secretion from phototrophs presented many hurdles in the translation of laboratory yields to scale. Butanol was the most robustly produced fuel in the laboratory, however, at volumes >100 L in outdoor photo-bioreactors, notable issues of product loss, poor microbe performance under fluctuating conditions, and general handling limitations were apparent. Aeration with large volumes of CO₂ (carbon source) and consumption from contaminants caused fuel losses at scale. Loss due to gas stripping (an issue for all volatile fuels) was solved using a custom photobioreactor with chemostat gas/medium pre-mixing. Contamination by bacteria was common outdoors at scale with limited options for low energy, high efficiency disinfection. Many laboratory strains do not have specialized metabolism to grow in extreme conditions (pH, temperature, salinity) used for bio-control in some algal cultures. As outdoor conditions differ vastly from those used to engineer strains in the laboratory, yields should be determined in controlled simulations of outdoor conditions prior to scale-up and may assist priming metabolism for daily variation cycles and adverse conditions.

Product characterization, fuel blending, and engine tests

In contrast to traditional biodiesel formulations from algal triacylglycerol oils, PHOTOFUEL sought to engineer direct secretion of liquid fuels from photosynthetic microbes. For market implementation, novel fuels and additives need to comply with chemical regulations and fuel standards, especially with regards to soot formation tendency. Proposed photofuels were, therefore, treated as blend additives to common fuels and tested in spark and compression ignition engines (SI and CI, respectively) at IFP Energies Nouvelles, Centro Ricerche FIAT, Neste, Volvo Trucks, and Volkswagen.

In SI engines, 1-butanol and isobutanol exhibited blend capacities into gasoline at high rates while fulfilling European standard for gasoline EN228. Soot formation reduction was noted, however, only isobutanol exhibited knock resistance of adequate value to serve in high octane petrol (HOP) fuels rated 102 research octane number (RON, knocking resistance indicator). 1-butanol has higher volumetric energy density and a lower vapor pressure than common ethanol additives and could be used in vehicle fleets with dedicated butanol sensors.

Hydrotreated vegetable oil (HVO) can be blended with diesel fuels used in CI engines and 1-butanol was also engine tested as a blend to this standard. 1-butanol addition to HVO-diesel drastically lowered the flash point, with blends more than 5 % no longer meeting European standard for diesel fuels (EN590), while soot formation was lowered below HVO-diesel alone.

Testing within PHOTOFUEL determined that isobutanol can be safely added as a blend to SI fuels, however, current CI HVO-diesel blends are not improved by 1-butanol addition. The engineering and production of 1-octanol in later stages of this project offers an alternative as this long chain alcohol could be used in CI engines.

Lifecycle assessment, cost and public perception

Lifecycle assessment (LCA) and techno-economic analysis (TEA) based on experimental data from upscaling and modeling indicated reduced environmental impacts of photofuels, but higher costs of production. Phototrophic cultures are generally dilute and often exhibit low engineered carbon flux to target products. Separation and purification of fuels (e.g. 1-butanol pervaporation and/or distillation) from dilute aqueous solutions strongly impacts process costs. Increasing carbon partitioning to product [11], choice of skimmable or products *in situ* extractable, the conversion of residues into co-products, waste re-use/valorization, low cost infrastructure (e.g. harvesting systems), increased automation (to reduce salary expenses), and reduced operating costs are key strategies to balance energy and cost economics. Other factors such as free CO₂ supply (waste from another process), nutrient recycling, renewable energies, and efficient photobioreactors will significantly improve carbon capture potential and environmental impacts of photofuels. Further technology efficiency improvements at all engineering levels and appropriate political framing will be necessary to practically realize photofuel processes.

In addition to LCA, understanding consumer acceptance of fuels produced by engineered microbes was investigated in PHOTOFUEL. Stakeholder interviews indicated that fuels from genetically modified photosynthetic microbes were perceived as environmentally advantageous compared to fossil and established biofuels across European countries [12]. Citizens are willing to pay more for such fuels if they can keep their environmental promises. The findings support public policy, regulatory decisions as well as financial support for increased research in this field.

Conclusion and outlook

The marriage of industrial process concepts with metabolic engineering of photosynthetic microbes will be a major challenge for the future development of sustainably produced fuels. Our work with multiple pathways in multiple species [8] indicated the uniqueness of each combination. There is no one-size-fits-all organism or product: hydrophobicity and molecular size impact efflux, necessitating customization of downstream processing and dictating energy/cost demands. Ideally, the product is not toxic to the host, not degradable by other organisms, and easy to separate. Medium-chain fuels, e.g. FAMES or octyl acetate, may be ideal due to extractability in two-phase systems. Solutions for respective *in situ* separation, milking

or organic phase self-formation should be integrated into bioreactor designs to enable low energy fuel recovery from dilute systems.

Contaminant-free biocatalytic production with high cell densities at large scale and low cost remains an outstanding challenge. Novel strategies to control the ecosystem inside reactors and cope with outdoor conditions must be developed already at the strain engineering stage, hand-in-hand with optimization of scale-up cultivation. To develop managed stable cultivation systems, synthetic consortia [13], or media/strain systems that exclude growth of other organisms should be systematically evaluated [14]. Robust thermophilic and halophilic strains like *Synechococcus* PCC 7002 may be less prone to contamination and can reduce costs.

The holistic approach of PHOTOFUEL enabled a targeted and critical discourse to steer engineering efforts at all levels. Genetic engineers took feedback from industrial culture experts, who interacted with process design specialists to design cultivation strategies for novel secreted hydrocarbon products. Engine testing and technological challenges in separation of hydrocarbons from aqueous cultures influenced genetic engineering strategies, and market and perception analysis impacted business case and scale planning. Although comprehensive studies are needed to identify optimal systems for cost-effective commercial biomanufacturing of fuels, PHOTOFUEL has set the foundations for their development and outlined some of the key issues to be addressed in the future.

220 **Declaration of interest**

221 Nothing to declare.

222

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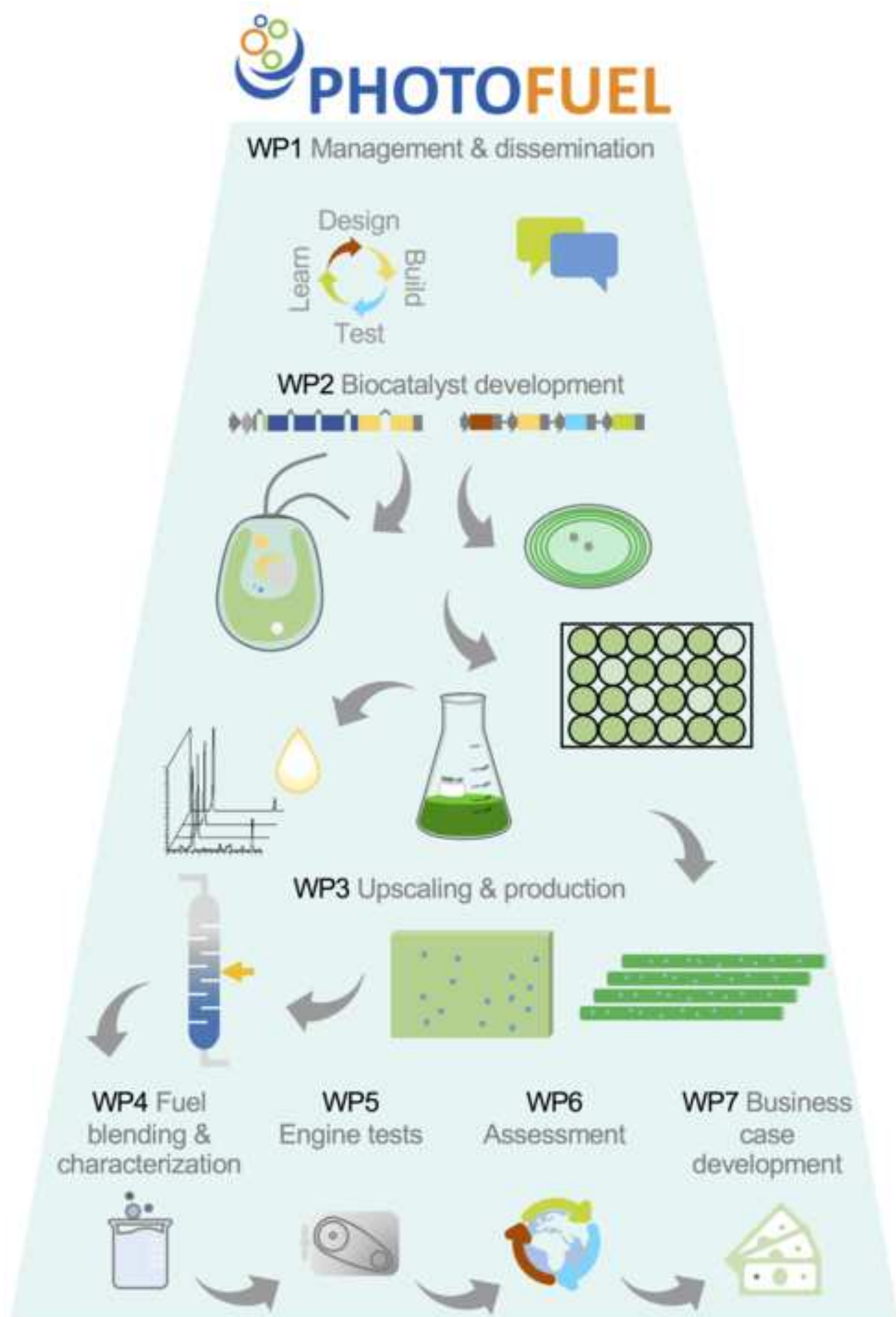
References

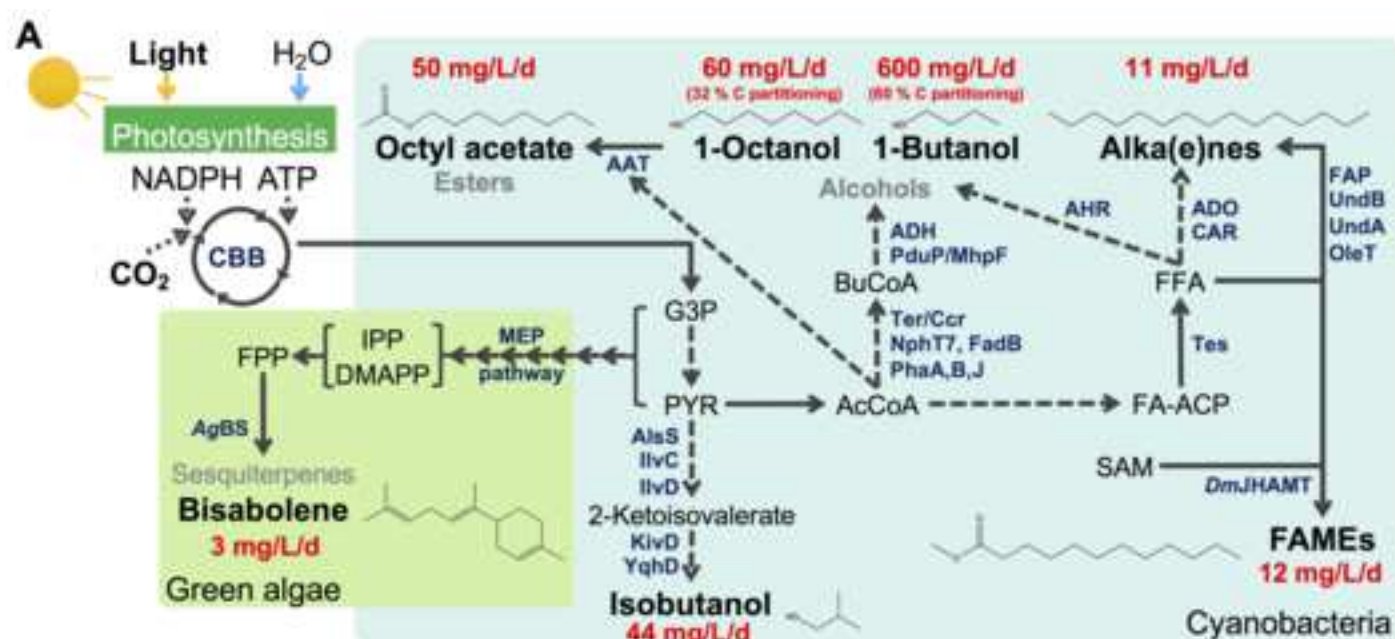
1. Miao R, Xie H, Lindblad P: **Enhancement of photosynthetic isobutanol production in engineered cells of *Synechocystis* PCC 6803.** *Biotechnol Biofuels* 2018, **11**:1–9.
2. Miao R, Xie H, M. Ho F, Lindblad P: **Protein engineering of α -ketoisovalerate decarboxylase for improved isobutanol production in *Synechocystis* PCC 6803.** *Metab Eng* 2018, **47**:42–48.
3. Miao R, Liu X, Englund E, Lindberg P, Lindblad P: **Isobutanol production in *Synechocystis* PCC 6803 using heterologous and endogenous alcohol dehydrogenases.** *Metab Eng Commun* 2017, **5**:45–53.
4. Liu X, Miao R, Lindberg P, Lindblad P: **Modular engineering for efficient photosynthetic biosynthesis of 1-butanol from CO₂ in cyanobacteria.** *Energy Environ Sci* 2019, **12**:2765–2777.
5. Yunus IS, Jones PR: **Photosynthesis-dependent biosynthesis of medium chain-length fatty acids and alcohols.** *Metab Eng* 2018, **49**:59–68.
6. Sattayawat P, Sofian Yunus I, Jones PR: **Bioderivatization as a concept for renewable production of chemicals that are toxic or poorly soluble in the liquid phase.** *Proc Natl Acad Sci U S A* 2020, **117**:1404–1413.
7. Yunus IS, Palma A, Trudeau DL, Tawfik DS, Jones PR: **Methanol-free biosynthesis of fatty acid methyl ester (FAME) in *Synechocystis* sp. PCC 6803.** *Metab Eng* 2020, **57**:217–227.
8. Yunus I, Wichmann J, Wördenweber R, Lauersen KJ, Kruse O, Jones PR: **Synthetic metabolic pathways for photobiological conversion of CO₂ into hydrocarbon fuel.** *Metab Eng* 2018, **49**:201–211.
9. Wichmann J, Baier T, Wentnagel E, Lauersen KJ, Kruse O: **Tailored carbon partitioning for phototrophic production of (*E*)- α -bisabolene from the green microalga *Chlamydomonas reinhardtii*.** *Metab Eng* 2018, **45**:211–222.
10. Miao R, Xie H, Liu X, Lindberg P, Lindblad P: **Current processes and future challenges of photoautotrophic production of acetyl-CoA-derived solar fuels and chemicals in cyanobacteria.** *Curr Opin Chem Biol* 2020, **59**:69–76.
11. 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**:205–216.
12. Varela Villarreal J, Burgués C, Rösch C: **Acceptability of genetically engineered algae biofuels in Europe: Opinions of experts and stakeholders.** *Biotechnol Biofuels* 2020, **13**:1–21.
13. Kazamia E, Aldridge DC, Smith AG: **Synthetic ecology - A way forward for sustainable algal biofuel production?** *J Biotechnol* 2012, **162**:163–169.
14. Loera-Quezada MM, Leyva-González MA, Velázquez-Juárez G, Sanchez-Calderón L, Do Nascimento M, López-Arredondo D, Herrera-Estrella L: **A novel genetic engineering platform for the effective management of biological contaminants for the production of microalgae.** *Plant Biotechnol J* 2016, **14**:2066–2076.

Figure captions

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	Challenges	Measures
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	Product evaporation	Product capture: in-line 2-phase extraction
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	Public acceptance	Environmental condition control (heating / cooling, artificial lighting) vs. choice of strain
		Advance knowledge on influence of variable outdoor conditions on production
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