ALGAESOL

Sustainable aviation and shipping fuels from microalgae and direct solar BES technologies

Starting date of the project: 01/05/2024
Duration: 36 months

= Deliverable: D3.4 =

P-BE-MA reactor - V1

Due date of deliverable: 31/10/2025
Actual submission date: 31/10/2025

Responsible WP: Nikola Medic, DTI.
Responsible TL: Sebastià Puig, T3.3, Universitat de Girona
Revision: V1

Dissemination level		
PU	Public	X
SEN	Sensitive, limited under the conditions of the Grant Agreement	
Classified R-UE/EU-R	EU RESTRICTED under the Commission Decision No2015/444	
Classified C-UE/EU-C	EU CONFIDENTIAL under the Commission Decision No2015/444	

Funded by The European Union

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101147112.

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DOCUMENT CONTROL

Document version	Date	Change
V0.1	25/09/2025	First Draft
V0.2	16/10/2025	Version for partners
V1.0	29/10/2025	Final version

VALIDATION

Reviewers		Validation date
Work Package Leader	Nikola Medic (DTI)	31/10/2025
Consortium reviewers	Daniele Molognoni / Ainhoa Cots (LEITAT)	31/10/2025
Project Manager	Xavier Ponte Font (NORCE)	31/10/2025
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DOCUMENT DATA

Keywords	Microbial electrosynthesis, microalgae
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DISTRIBUTION LIST

Date	Issue	Recipients
16/10/2025	V0.2	Project Coordinator, WG Leaders
31/10/2025	V1.0	EC, Project Partners

Executive summary

The present document (Deliverable D3.4 – P-BE-MA V1), reports the outcomes of WP3-Task T3.3, which defined the setup structure and performance achievements of the first version of the photo, bioelectrochemical microalgae (P-BE-MA) reactor to achieve neutral lipids for sustainable aviation fuels production. This executive summary outlines the second deliverable relative to WP3 of the ALGAESOL project (D3.4), which focuses on the development of the core bioelectrochemical technology and microalgae cultivation unit developed in WP3.
Deliverable D3.4 was prepared by UdG. The outcomes of task T3.3 reported in this document will be relevant in the further definition of the process streams and for all tasks related to modelling and simulation, as well as T2.4 and T3.4 concerning the processing of the microalgae biomass produced.

Abbreviations

The abbreviations relevant for WP3-T3.3, used in the ALGAESOL Deliverable D3.4 are listed in the table that follows.

Table 1: Abbreviations used in this document.

Abb.	Description	Abb.	Description
AN	Anode	MA	Microalgae
BES	Bio-Electrochemical System	MES	Microbial electrosynthesis
CAT	Cathode	Mx	Project Month x
CEM	Cationic exchange membrane	PA	Photoanode
CE	Counter electrode	SAF	Sustainable Aviation Fuel
DSA	Dimensionally stable anode	VFAs	Volatile Fatty Acids
EC	Electric conductivity	WE	Working electrode
EU	European Union	WP	Work Package
FE	Faradaic efficiency	WPx-Ty.z	Work Package x Task Ty.z
GA	Grant Agreement	WT	Wildtype
GHGs	Green House Gases	WW	Wastewater

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1. Introduction

In just over a century, human activity significantly played a part to the constantly increasing greenhouse gas (GHG) emissions worldwide (Melillo et al., 2009). Researchers' interest in finding new strategies for GHG emissions mitigation increased sensibly and led governments to encourage to increase the energy efficiency of productive processes, promote the use of non-fossil fuels, and enhance the capture and storage of carbon dioxide (CO_2) as well as its valorization. Bioelectrochemical systems (BES) are a flexible platform to perform carbon bio-electro recycling into added-value products. Specifically, microbial electrosynthesis (MES) is a process that uses electroactive microorganisms and electrical energy to convert carbon dioxide (CO_2) into value-added products such as methane, hydrogen, volatile fatty acids (VFAs), and other organic compounds. With the increasing interest on resource recovery and circular economy, MES appear like an appealing technology to produce valuable compounds, among all, biofuels. A large variety of substrates can be used for (bio)electrosynthesis of value-added products. The choice of substrates for the (bio)electrochemical conversion of wastes into value-added chemicals has gradually been expanded to various wastewaters, solid wastes, and waste gas (Kong et al., 2020). Furthermore, MES has several advantages over traditional bioprocessing methods, including the ability to use renewable energy sources surpluses, such as solar or wind power, to provide the electrical energy required for the process, and to store the so-converted carbon into gaseous or liquid fuels (Dessì et al., 2021). Steering production towards the most valuable compounds at high purity, and downstream processes for certain compounds unfortunately are options not always viable and/or economically sustainable (Prévoteau et al., 2020). Thus, coupling BES with other biological processes appears to be a promising strategy for their exploitation (Thulluru et al., 2023).

Because of their high carbon content (including carbohydrates, proteins, and lipids), fast growth rate, short life cycle, and high tolerance to different conditions (in terms of temperature, pH, salinity, and light intensity), microalgae are getting a lot of attention. Microalgae are considered as a resource for greenhouse gases (GHG) emissions mitigation, plus an essential feedstock for microalgal biorefinery products, such as biofuels (i.e. biodiesel through oil transesterification) and food and feed goods (Okeke et al., 2022). Autotrophic microalgae cultivation relies exclusively on light and inorganic carbon (typically CO_2) as energy and carbon sources, making it a sustainable option for CO_2 capture and nutrient removal in wastewater-based systems. However, autotrophic microalgae growth is strongly dependent on illumination conditions and photosynthetic efficiency. Heterotrophic metabolisms, which involve the use of organic carbon sources, have been found to result in higher lipid productivities and more suitable quality compared to photoautotrophic conditions. The key factors affecting the lipids production in heterotrophic microalgae include the presence of organic carbon sources, cell growth rates, final cell concentration, and oil accumulation capacity of the cells. Additionally, factors such as nitrogen limitation, phosphate limitation, silicon limitation, control of pH, and low temperature can be used to increase oil accumulation in heterotrophic microalgae (Zhang et al., 2013). Mixotrophic cultivation, in contrast, combines photosynthesis with the uptake of organic carbon sources (e.g., acetate, glucose), which can significantly enhance growth rates and biomass yields, particularly under suboptimal light regimes, and at the same time reduce contamination of the culture.

Coupling BES with microalgae is an interesting option to enhance resource recovery by applying a biorefinery approach through the production of biofuels and other value-added products (Bolognesi et al., 2022; Sevda et al., 2019). Heterotrophic and mixotrophic microalgae, though not directly exploiting CO_2, can use acetate and other short chain fatty acids produced in MES for their growth, accumulating lipids in their cells, with no need of continuous light exposure (Ren et al., 2019). Nonetheless, there are still challenges to be addressed in this process, such as the optimization of the microalgae-BES system, the development of efficient and scalable photobioreactors, and the identification and cultivation of suitable microorganisms to enhance fuel precursors production.

One of the major challenges for microalgae integration in MES is membrane fouling, which reduce the lifespan of reactors operated and their performances (Pasternak et al., 2022). For this reason, maintaining separate units in a sequential process is the most likely commercial outcome of BES-Microalgae systems.

In this context, ALGAESOL ("Sustainable aviation and shipping fuels from microalgae and direct solar BES technologies") is a publicly funded Horizon Europe project (GA No. 101147112), that aims to develop and evaluate

innovative solutions for the sustainable conversion of sunlight into fuels. ALGAESOL will advance the current state-of-the-art by creating and consolidating new value chains for shipping and aviation biofuels based on micro-algae and direct solar renewable fuel technologies. Among the core technologies developed in the ALGAESOL project, the P-BE-MA consists of a photo-bioelectrochemical and a microalgae cultivation unit. This two-stage process is based on coupling a photo-bioelectrochemical system for acetate production, equipped with a photoanode to reduce energy consumption (P-BE-) and a mixotrophic/heterotrophic microalgae cultivation unit (MA) to perform microalgae-enhanced CO_2 conversion into neutral lipids.

The P-BE-MA reactor is a core component of the project's technical work on sustainable aviation fuels (SAF) production from microalgal neutral lipids. To achieve sustainable fuels production, working conditions optimization of both BES reactor and microalgae cultivation unit have to be optimized. The present deliverable D3.4 aims at (1) optimize the continuous operation of a BES reactor for CO_2 bioelectrorecycling into acetate, maximizing production rates and minimizing energy consumption and (2) assess the performance of the wildtype microalgae *Chlorella sorokiniana* with real, raw BES effluent to identify the optimal cultivation condition.

Deliverable D3.4 corresponds to the first functional prototype (V1) and documents:

- The concept and description of the experimental setup;
- The assembly of the setup (bioelectrochemical system and microalgae reactor);
- Qualitative performance and degree of completion of the objectives;
- Planned improvements for the second version of the prototype (photoanode installation, improved microalgae, V2).

2. Experimental setup

The design of the P-BE-MA V1 reactor integrates two separate units: a **bioelectrochemical system** (**BES**) for CO_2 conversion into acetate, and a **microalgae cultivation unit** (**MA**). This design choice relies on the fact that a modular approach allows more flexible scalability of the two units and eases the setups maintenance in laboratory testing conditions. The first version of the setup does not include a **photoanode** (**PA**), which is currently under development, and it will be installed in the P-BE-MA V2.
No sensitive or unpublished parameters (e.g., detailed operational conditions applied, or control logic) are disclosed in this public version of the deliverable, but the relevant results produced in WP3-T3.3 will published in a peer-reviewed open access publication in the following months and made available to the broad public.

2.1. Bioelectrochemical system

The bioelectrochemical setup selected to carry out the activities connected to WP3-T3.3 consisted of two double-chambered, low-gap commercial electrochemical reactors (Electro MP Cell®, ElectroCell, Denmark), inoculated at the cathode side with acetogenic biomass from a parent BES reactor at UdG facilities. Figure 1 presents the experimental setup installed at UdG facilities. Cathodic and anodic chambers were separated by a cationic exchange membrane (CEM), and their working volumes expanded through the use of recirculation buffer tanks. Continuous mixing was granted by using a multichannel peristaltic pump (100 L d^{-1}, Lab M1, Shenchen peristaltic pump, China). The cathode (carbon felt, 0.01 m^2 + stainless steel collector) and the anode (dimensionally stable anode, 0.01 m^2, DSA®) were respectively the working (WE) and counter electrode (CE). No reference electrode was installed in the BES setup. The BES was operated in a two-electrode configuration, with a potentiostat (Bio-Logic SP50, France) controlling the current provided (5-15 A m^{-2}) and recording the cell voltage and power consumption every five minutes. Experiments were conducted at room temperature (25 ± 3°C), with a thermal blanket installed to prevent excessive temperature fluctuations. Synthetic mineral medium as in Rovira-Alsina et al. (2019) was used as both anolyte and catholyte and replaced manually (batch testing phase) or through multiple pulses throughout the day (semi-continuous phase). Cathodic chambers of both cells were fed with CO_2 gas (99.9%, AirLiquide, Spain) as the only carbon source. The setup was complemented by dissolved CO_2 probes (Mettler Toledo) to monitor the available CO_2 concentration every 5 minutes. The system under this configuration was operated since M4.

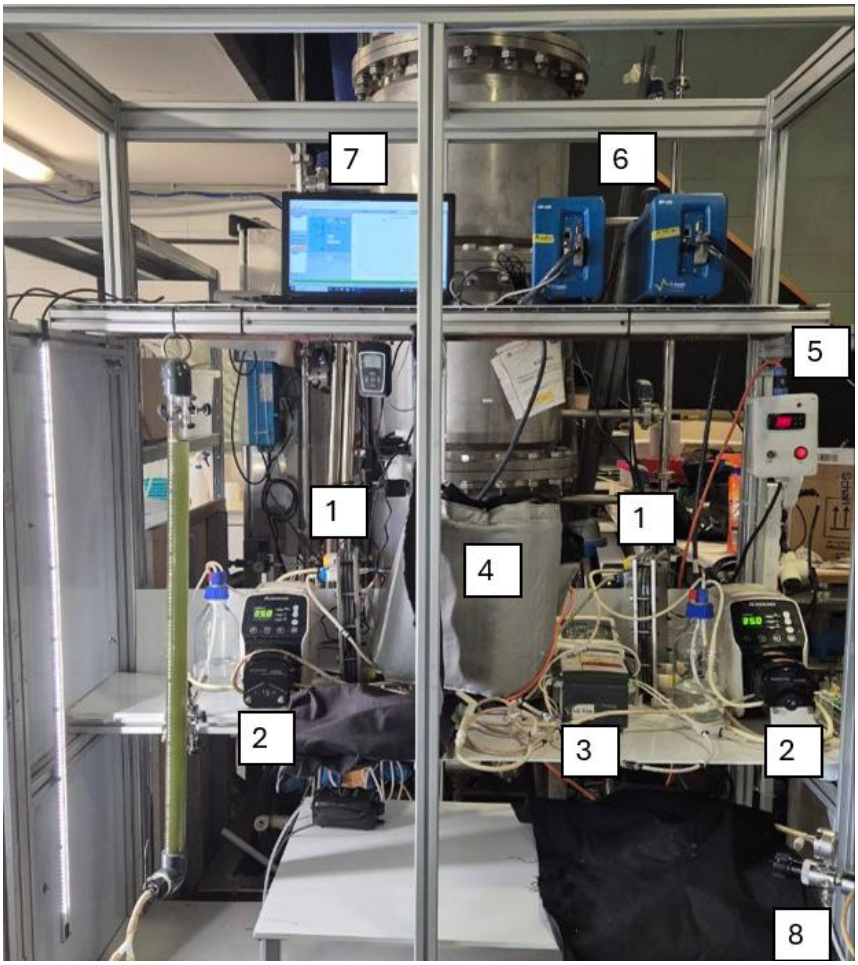

Figure 1: BES reactor setup (P-BE-MA V1). The first version of the setup does not include the photoanode (PA). 1) BES; 2) recirculation pumps; 3) feeding pumps; 4) thermal blanket (heating cathodic recirculation tanks); 5) temperature control; 6) potentiostats; 7) laptop; 8) CO_2 tank.

2.2. Microalgae cultivation unit

The microalgae cultivation (Figure 2) unit was manufactured by AQUALGAE (Spain), model FBR3C (n°115/2025). The system consists of a metallic structure made of AISI316 stainless steel, supporting three methacrylate columns (Ø110 mm) which served as independent cultivation units. Each column can allocate between 5 and 10 L. A LED light (C65, Valoya) has been installed behind each column to provide controlled illumination under selected light intensities up to 200 µmol photons m^{-2} s^{-1}. The setup includes a 200 W water heater (to control temperatures in the range 20-34 °C) and a combined pH/temperature probe for efficient process monitoring. Control and automation are managed through a main electrical panel, complemented by a secondary electrical panel equipped with digital flow sensors for the measurement and injection of CO_2. The system also features a manifold for the supply of water and/or medium to the columns, as well as a flowmeter allowing the regulation of the air flow (1 to 10 L min^{-1}) to prevent sedimentation of the microalgae. CO_2 injection can be either timed or pH-regulated by setting a threshold to prompt flowmeters activation.

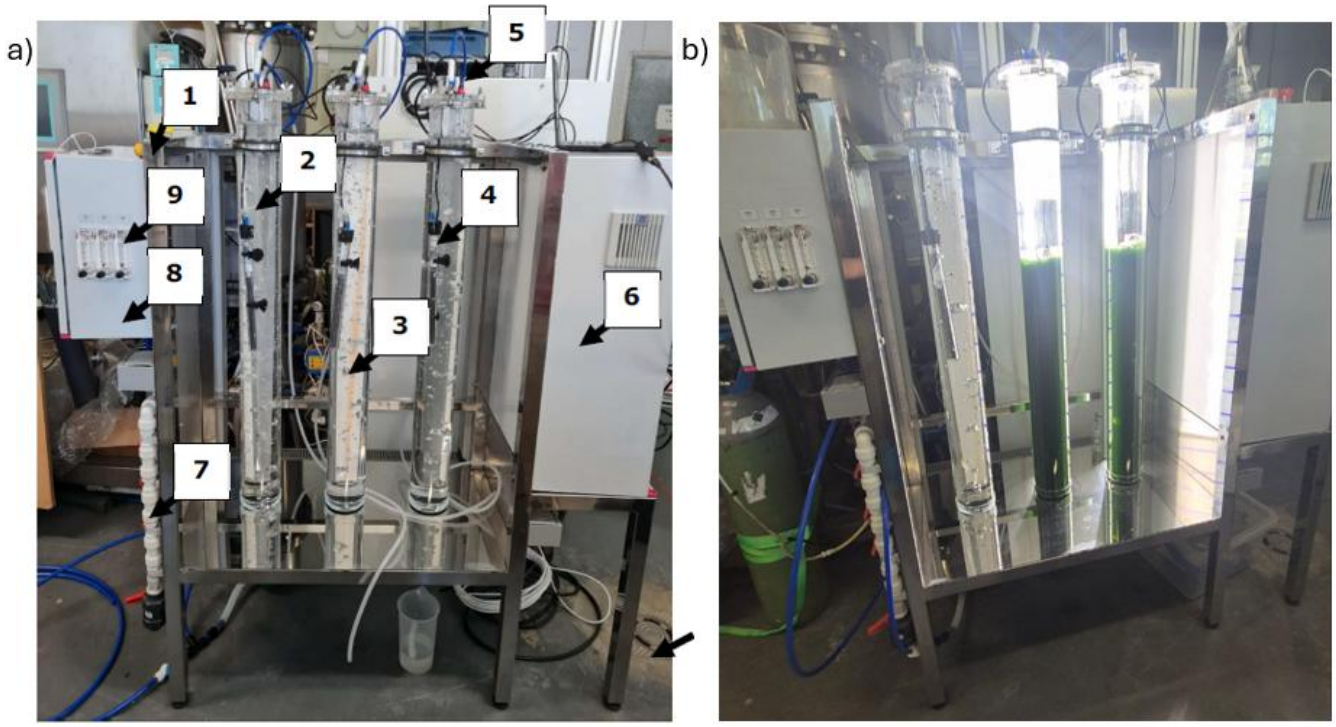

Figure 2: Microalgae cultivation unit setup (P-BE-MA V1). a) details of components: 1 – Metallic structure, 2 – Methacrylate column for cultivation, 3 – LED, 4 – water heater, 5 – pH/temperature probe, 6 – Main electrical panel, 7 – Hydraulic system, 8 – Secondary electrical panel (CO_2 supply), 9 – flowmeter. b) microalgae cultivation unit in operation.

The control and monitoring system (Figure 3) includes an electrical voltage indicator light and a 7" touch console that allows comprehensive control and monitoring of the FBR3C. The process is managed through a JUMO Aquistouch S controller, which provides precise regulation of operational parameters. The system also features a main ON/OFF switch for the electrical panel. In addition, the gas management section incorporates digital flow sensors for accurate measurement and regulation of CO_2 flow, along with a gas solenoid valve that enables automated and safe gas injection, contributing to the overall efficiency and stability of the system.

Figure 3: Control panel of the microalgae cultivation unit. 1- indicator light, 2- 7" touch console, 3- JUMO Aquistouch S controller, 4- Main ON/OFF switch, 5- Manufacturer marking plate.

2.3. Envisioned overall process

The overall process relies on full exploitation of sunlight and CO_2, in order to maximize microalgae productivity and minimize global energy consumption. The BES will produce acetate throughout the day, exploiting either solar energy surpluses as a source of energy (connected to a solar panel) or direct solar energy using a photoanode implemented at the anodic side (available for P-BE-MA V2). The microalgae cultivation unit will be exposed to sunlight during the day, prompting phototrophic growth of *C. sorokiniana* and full exploitation of sunlight and CO_2. The effluent rich in acetate generated by the BES during the day will be fed to the microalgae cultivation unit at night, to prompt microalgae heterotrophic growth. Such microalgae trophic mode has been implemented for the wildtype (WT) and it will be applied also to the improved strain in the P-BE-MA V2.

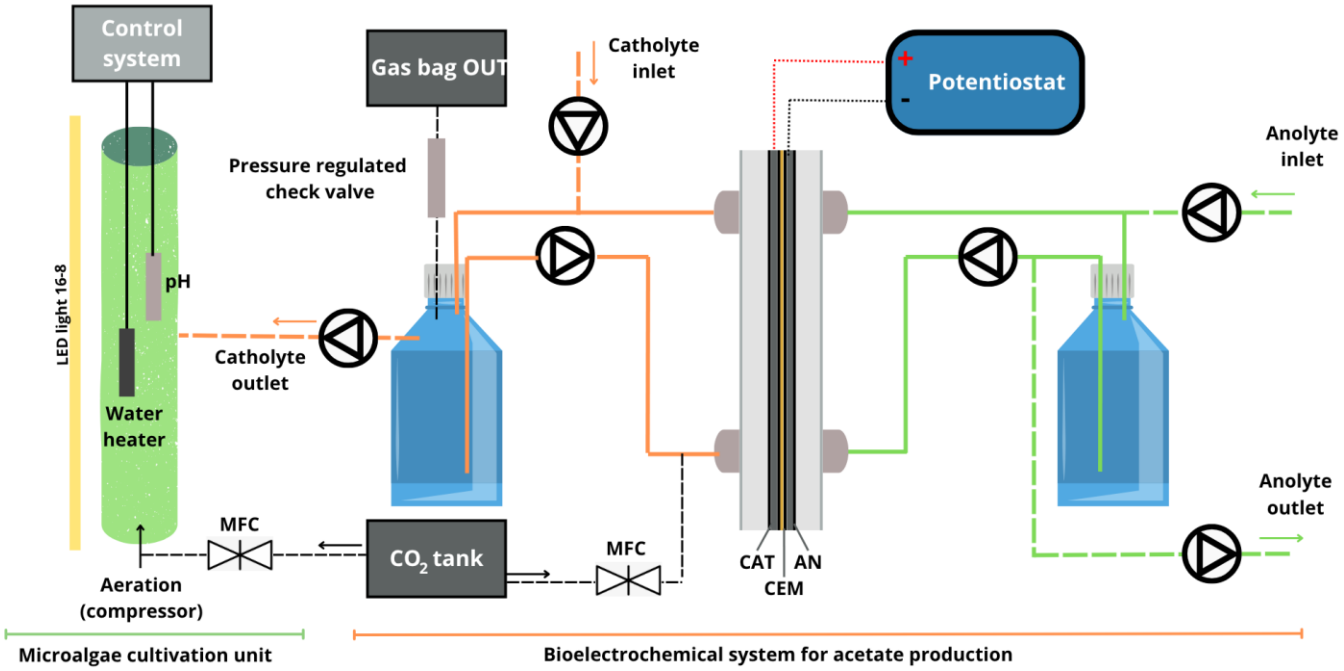

Figure 4: P-BE-MA V1 overview schematic. CAT: Cathode. AN: Anode. CEM: Cationic exchange membrane. MFC: mass flow controller. In the first version of the prototype does not include a photoanode, which will be installed in place of the conventional anode mounted in the V1.

2.4. Analytical methods

BES Reactor. The BES activity was steered by setting a fixed current between cathode and anode, and monitoring voltage (V), power (W) and charge consumed (C) every 5 minutes. During the whole operational period, optical density (OD_{600}), electric conductivity (EC), pH, temperature, and cathodic CO_2 dissolved concentration were monitored in the liquid sample collected or in-line (in the case of dissolved CO_2). Gas and liquid samples were collected three times per week to control the performance of the BES in terms of volatile fatty acids (VFAs) production, following the procedure described in Bolognesi et al. (2022). The cathodic pH in the liquid samples was measured at each sampling and corrected manually at need to be maintained in the optimal range 5 to 6.5.

Microalgae cultivation. Optical density, to monitor chlorophyll (OD_{680}) and microalgae (OD_{750}) growth, electric conductivity (EC), pH, temperature, cell dry weight (through measure of total suspended solids), cell counting and VFA content have been measured consistently to evaluate the performance of the *C. sorokiniana* (wildtype) under autotrophic, heterotrophic and mixotrophic conditions, and assess its possible growth with BES effluent as a substrate. Potential contamination of the culture has been monitored through microscopy and the measure of OD_{600}, and through the shipment of samples (fresh and/or frozen) to DTI for further analysis. Autotrophic and

mixotrophic tests have been performed at 100 µmol photons m^{-2} s^{-1} light intensity under a 16/8h light/dark profile, while during heterotrophic tests the reactors have been covered with aluminum foil to prevent light intrusion.

3. Summary of results

3.1. Reactors design and assembly

The experimental setup has been assembled and installed at UdG facilities. After successful hydraulic testing and inoculation, several essays have been performed in both BES reactors and microalgae cultivation units separately, in order to assess the optimal operational condition. This study will serve as a basis for the connection of the two units, and as a baseline conditions prior to its further improvements foreseen for the P-BE-MA V2. A summary of the achievements of both BES reactor and microalgae cultivation unit is presented in the next paragraphs (3.2. and 3.3.).

3.2. Preliminary BES optimization and testing results

The primary objective of the BES operation was to generate acetate at concentrations and production rates sufficient to ensure regular and stable feeding of the downstream microalgae cultivation unit. To this end, the BES was operated under conditions aimed at maximising acetate production, with particular attention to the applied current density and operational regime. Complete quantitative results (in terms of production rates, coulombic efficiency, and carbon conversion rates) will be reported in internal reports and upcoming peer reviewed scientific publications. A broad outline of the parameters range used for system optimization in the first year of operation has been included in Table 2.

Early-stage qualitative tests demonstrated:

- Successfully identified two operational strategies (one in fed-batch cultivation mode, one in semi-continuous mode) to be applied in long-term experiments.
- Stable acetate production after 1 month of operation, with production rates up to 100 g m^{-2}cat d^{-1} in the best tested conditions, and consistently over 40 g m^{-2}cat d^{-1}.
- Energy consumption for acetate production lower than 13 kWh kg-Hac^{-1} in some of the conditions tested.
- High average faradaic efficiency (FE) 93.4 ± 13.8%, associated to a carbon conversion efficiency to Hac up to 50%.
- Stable operation with minor maintenance events, during one year.

Table 2. Parameters used for the BES reactor optimization.

Parameter	Variation timeline	Range	Function
Current density [A m^{-2}]	M6-ongoing	5-15	Increase H_2 availability.
HRT [d]	M9-ongoing	10-20	Increase the volume of BES effluent produced.
CO_2 injection	M6-M9 (batch)	Daily – Every 2 days	Increase CO_2 availability and prevent its limitation with more frequent provision.
	M15-Ongoing (intermittent)	Number of pulses per day – gas flow	Increase the CO_2 availability and maintain it to the desired (optimal) concentration in the reactor.
CO_2 overpressure (batch)	M6-M15	250 - 500 mbar	Increase dissolved CO_2 Concentration.

As the acetogenic biomass gets acclimated to operational conditions applied over time, it is not excluded that there will be further margin of improvement of the semi-continuous strategy identified in this P-BE-MA V1 in M19-M30. Alternation of ON/OFF current provision periods associated with the PA implementation might prompt a different approach in terms of current/feeding/operational conditions.

3.3. Microalgae cultivation

Following the production of acetate in the BES, preliminary microalgae cultivation assays were conducted to assess the suitability of the BES effluent as a carbon source for *C. sorokiniana*. These tests aimed to determine the optimal proportion of BES effluent to be supplied to the microalgae cultivation unit, ensuring adequate nutrient availability while preventing potential inhibitory effects. Different feeding ratios were evaluated to identify operational conditions supporting stable growth performance and efficient acetate assimilation by the microalgal biomass. The outcomes of these preliminary tests served as the basis for defining the feeding strategy that will be applied in subsequent cultivation trials and in the conceptualization of the strategy for continuous process implementation. So far, only microalgae growth has been prioritized as a parameter over lipid quantity and quality. The culture is permanently maintained in autotrophic cultivation to prevent external contamination until testing. Heterotrophic and mixotrophic tests were performed at different initial concentrations of acetate (1-6 gHac $g_{biomass}^{-1}$) monitoring culture status and biomass growth for up to 3 days.
Preliminary results demonstrated promising growth rates, with biomass production reaching up to 3 g L^{-1} d^{-1} when using acetate derived from the BES effluent. These findings confirmed the feasibility of coupling the BES with the microalgae cultivation process and guided the definition of the feeding strategy applied in subsequent trials. Heterotrophic tests with concentrations superior to 6 gHac $g_{biomass}^{-1}$ led to evident contamination of the biomass and death of the microalgae culture, both in filtered and unfiltered BES effluent essays. Alternation of photo and heterotrophic trophic modes helped maintaining under control bacterial contamination of the culture. Further attention will be posed to contamination control strategies in close collaboration with DTI.

4. Future actions

The P-BE-MA V1 prototype confirms the feasibility of the proposed reactor configuration. In M19-M30, further improvements will foresee:

- Further increasing the acetate production of the BES reactor, by applying continuous CO_2 feeding and testing higher current densities/profiles.
- Coupling the BES's biocathode for acetate production with the photoanode developed in T2.1.
- Perform medium to long-term microalgae cultivation experiments with BES effluent.
- Perform short-term, medium-term and long-term cultivation experiments with the improved *C. sorokiniana* strain and/or with controlled substrate limitation to enhance lipid production.
- Improving energy efficiency through current provision strategies (intermittent, photoanode installation).

5. Conclusions

This report describes the P-BE-MA reactor features and performance up to M18, summarizing the key findings and achievements. The two units composing the P-BE-MA have been installed at the UdG facilities, and operated independently (V1) with the objective of finding the optimum and sustainable coupling strategy to be implemented in the P-BE-MA V2. High acetate production rates (up to 100 g m^{-2} d^{-1}) and low energy consumption (<13 kWh $kgHac^{-1}$) have been achieved through variation of operational conditions (current density, HRT, CO_2 provision strategy). *C. sorokiniana* cultivation with BES effluent has also been proven successful in short term cultivation trials (up to 3 days). More detailed results, in accordance with the public nature of the present deliverable, will be included in a peer-reviewed open access publication.

6. Degree of progress

The main experimental objectives connected to the optimization of the BES-microalgae system were successfully achieved. The (P)-BE-MA V1 (considered as the BES for acetate production and the microalgae cultivation unit) is fully operational and demonstrated stable acetate production under both batch and semi-continuous modes, reaching concentrations suitable for feeding the downstream microalgae cultivation unit. Preliminary microalgae cultivation assays were completed, confirming the compatibility of the BES effluent as a carbon source, and identifying optimal feeding ratios to support efficient mixotrophic/heterotrophic growth. These achievements represent a significant step toward the validation of the integrated P-BE-MA system. Ongoing work focuses on

refining operational parameters, improving process stability, and preparing for longer-term microalgae cultivation trials. The deliverable has been completed on schedule, describing the P-BE-MA prototype used in WP3-T3.3 and highlighting the key results.

7. Dissemination level

The dissemination of information related to the ALGAESOL project deliverable 3.4 is categorized as Public, with no restrictions under the conditions of the Grant Agreement. The results included in this report will be published in extended form in a peer-reviewed, open access journal as well as made available in the project repository in due time. Careful consideration will be given to the timing and manner of dissemination of such information, to safeguard sensitive data while maintaining compliance with all relevant regulations and agreements.

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