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D3.2. Selection of the final device

Artificial PHOTOsynthesis to produce FUELs and chemicals: hybrid systems with microorganisms for improved light harvesting and CO2 reduction

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EXECUTIVE SUMMARY

This deliverable presents the results of Task 3.3, focusing on the selection of the final device to be upscaled as part of the Horizon Europe **Photo2Fuel** project. This study compares the performance of two biohybrid systems for sustainable chemical production: methane generation using Methanosarcina barkeri and acetic acid synthesis utilising Moorella thermoacetica.

The acetic acid system achieves higher yields and operates with a more straightforward reaction pathway, offering greater efficiency. Additionally, it avoids the complexity and energy losses in comparison with the methane production system. The acetic acid system also demonstrates stronger scalability, making it more suitable for industrial applications.

Environmental, economic, and social assessments using the Multidisciplinary Design Optimisation methodology further confirm that the acetic acid system outperforms the methane system. It is more cost-effective and sustainable, offering a scalable solution for large-scale chemical production.

1. INTRODUCTION

1.1 DESCRIPTION OF THE DOCUMENT AND PURSUE

This deliverable aims to provide a comprehensive analysis of two biohybrid systems for sustainable chemical production: methane generation using Methanosarcina barkeri and acetic acid production with Moorella thermoacetica. The document focuses on evaluating the performance of these systems with respect to key factors such as efficiency, scalability, and economic viability. Through this evaluation, the deliverable justifies the decision to prioritise the acetic acid system for scaling up, based on both experimental results and Multidisciplinary Design Optimisation (MDO) analysis.

1.2 WPS AND TASKS RELATED TO THE DELIVERABLE

This deliverable is directly linked to the activities and objectives of WP3 – Modelling, which focuses on the modelling, optimisation, and validation of the two Photo2Fuel systems.

Links to Other Work Packages and Tasks:

- Laboratory results from WP2 were critical in identifying the limitations of the methane production pathway and the advantages of the acetic acid system.
- Task 4.2 within WP4 is directly linked to this deliverable. Following the selection of the Moorella thermoacetica-based system, Task 4.2 will focus on assembling a reactor for the upscaled device. This reactor will incorporate a luminescent solar concentrator paired with the hybrid microorganism-organic semiconductor system to optimise light absorption and enhance the acetic acid production process.

1.3 OBSERVATIONS RELATED TO THE DELIVERABLE

This deliverable was delayed since it needed experimental details from WP2 to justify the election of the system that would be upscaled (acetic acid / methane) to properly justify this decision based on science.

2. LABORATORY RESULTS AND KEY FINDINGS

Biocatalytic systems have gained significant attention as sustainable alternatives for chemical production. Among these, hybrid systems combining biological entities with synthetic components represent a promising approach to convert solar energy into valuable chemicals. A critical component of these biohybrid systems is the use of **organic photosensitizers**. These materials are metal-free and capable of absorbing light, typically in the visible or near-infrared spectrum, and transferring the resulting energy or electrons to other molecules or catalysts to drive chemical reactions. Organic photosensitizers are highly customizable, allowing precise tuning of their optical properties, making them ideal for integration into biohybrid systems[1].

During the **Photo2Fuel** project execution, three categories of organic photosensitizers were synthesized and evaluated for their compatibility with the two microbial systems (M. barkeri and M. thermoacetica):

- Polymer-based nanoparticles (Pdots): These include nanoscale particles composed of polymers, with unique optical and electrical properties.

- Small molecule-based nanoparticles (POZ-M NPs): These are nanoscale particles based on organic molecular structures, valued for their optical properties in the visible spectrum.

- Carbon dots (Cdots): These are ultra-small nanoparticles (1 – 10 nm) composed primarily of carbon, often incorporating heteroatoms such as nitrogen, sulphur, or oxygen. They are prized for their compact size and excellent biocompatibility.

Each category was tested for its ability to integrate with the two microbial systems to drive chemical transformations. A detailed description of these materials, their synthesis, and testing is provided in **Deliverable 2.2: Final report of the hybrid devices: testing, characterisation, and efficiency**.

The two systems were tested under controlled conditions to determine their suitability for scaling. A combination of optimisation protocols, including modifications to growth media, physical conditions, and integration with light-harvesting materials, was applied. The key experimental parameters and findings are detailed below.

2.1 PERFORMANCE ANALYSIS OF M. BARKERI

2.1.1 Growth and Media Optimisation

Methanosarcina barkeri was cultured in heterotrophic DSM120 media[2] (pH 6.8) under a gas atmosphere of 80% H_2 and 20% CO_2 at 2 bar absolute pressure and 37°C. Cells were harvested by centrifugation (5000 rpm for 5 minutes) and washed three times with autotrophic DSM120 medium (it refers to the heteroptrophic DSM120 media, without using yeast, casitone and $NaHCO_3$), adjusting the pH to 7.0.

2.1.2 Photocatalysis and Experimental Conditions

For photocatalytic experiments, an autotrophic DSM120-based medium was further developed and tested under varying conditions (e.g., pH, redox potential, and temperature) to ensure stability and cell viability. Optimisations were performed to promote a single-cell or small-cluster morphology, enhancing surface area interaction between the archaea and the photosensitizers. This morphology was critical to maximise electron transfer efficiency in biohybrid systems.

Photocatalytic measurements were conducted under a CO_2 (30%) and N_2 (70%) atmosphere. The following actions were considered:

- Cell Density: Adjusted to OD600 0.2 after homogenising samples to achieve uniform single-cell morphology.
- Photosensitizers: Tested Pdots, POZ-M NPs, and Cdots.
- Reaction Conditions: The reaction mixture included 11.4 mM cysteine, a 0.25 mM redox mediator (methyl viologen for Pdots and Cdots, DQ-OH for POZ-M NPs), and a 2:1 media-to-gas ratio in a total volume of 4 mL. The pH was maintained at 7.0, with samples vacuumised, sparged for 5 minutes, pressurised to 3 bars, and incubated at 37°C.
- Illumination: Samples were exposed to full-spectrum LED light (2.2 mW/cm²) for six days to activate the photosensitizers.

The efficiency of the microorganism-organic semiconductor systems for methane production was analysed using gas chromatography. Figure 2.1 shows the corresponding GC chromatograms.

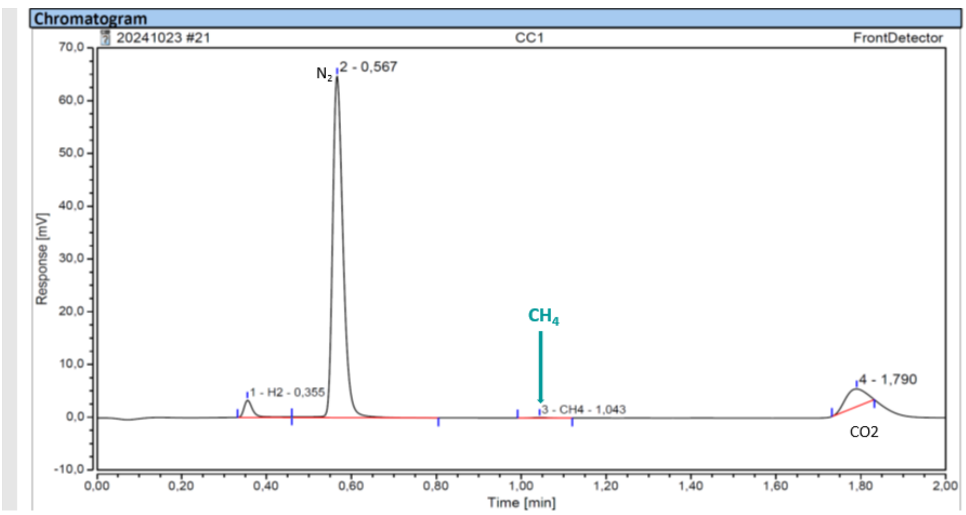

Figure 2.1. Sample chromatograms for biohybrid assemblies – Cdots as photsensitizers.

Methane production was only detected in the biohybrid systems that used Cdots as photosensitizers. Gas chromatography analysis showed methane yields with a relative area percentage (Rel Area % CH_4) of 0.22%, a value deemed insufficient for scaling the process.

Other photosensitizers, such as Pdots and POZ-M NPs, failed to produce measurable methane, likely due to incompatibility with the metabolic pathways of M. barkeri or inadequate electron transfer efficiency. Mechanistic insights suggest that hydrogen, generated as a side product by Cdots, was metabolised by M. barkeri to produce methane. However, **the overall system efficiency remained too low for industrial applications**.

The **low methane yields** and the **dependency on specific photosensitizers** (**Cdots**), highlighted significant limitations in the scalability of the M. barkeri-based system. These challenges caused a transition in focus toward optimising the acetic acid production system involving M. thermoacetica.

2.2 PERFORMANCE ANALYSIS OF M. THERMOACETICA

2.2.1 Growth and Media Optimisation

Moorella thermoacetica was cultured in autotrophic media optimised for thermophilic growth, with incubation at 55–60°C. The gas phase comprised 20% CO_2 and 80% H_2, pressurised to 3.9 bars. Growth dynamics were assessed using OD600 measurements, and acetic acid production was quantified using high-performance liquid chromatography.

2.2.2 Photocatalysis and Experimental Conditions

Two different strains of M. thermoacetica were used for the photocatalytic experiments: the ATCC 39073 strain (**ICCAS**) and the DSM 2955 strain (**TZE**). **ICCAS** conducted the initial testing and optimization of Pdots along with M. thermoacetica during the M0 – M24 period, while **TZE** used optimised conditions to reproduce results with the best-performing Pdots and added POZ-M NPs and Cdots to their studies. Both groups followed similar protocols[3] for bacterial growth, biohybrid preparation, and photocatalytic conditions. **TZE** also optimised conditions such as centrifugation speed, reaction volume, and incubation times for improved bacterial viability and efficiency.

TZE's experiments showed that **POZ-M NPs had the highest efficiency for electron transfer**, and further optimization studies adjusted factors like nanoparticles concentration and pressure.

For a more advanced and detailed description, please review **Deliverable 2.2: Final report of the hybrid devices: testing, characterisation, and efficiency**.

3. SUMMARY OF MDO METHODOLOGY

The **Multidisciplinary Design Optimisation** (**MDO**) methodology[4,5,6] applied in this study was thoroughly defined in **Deliverable 3.1: Definition of the MDO Problem** and further developed in **Deliverable 5.2: Sustainability Assessment of the Two Hybrid Systems**. Essentially, MDO integrates economic, environmental, and social criteria to identify the most sustainable solution.

The implementation of the MDO employed the following tools and frameworks:

1. Technique for Order Preference by Similarity to Ideal Solution (**TOPSIS**) Algorithm: Programmed in Python to facilitate a flexible and efficient evaluation of the systems.

2. Data Processing Tools: Input data for environmental, economic, and social indicators were processed using Excel and Python Pandas libraries.

3. Visualisation Tools: Results were visualised using the Matplotlib and Seaborn libraries to generate clear, interpretable figures and insights to support decision-making.

4. Data Validation: Environmental impacts were calculated using Life Cycle Assessment (LCA) methods. Economic and social indicators were derived from experimental data and validated against existing databases.

The TOPSIS approach was used to evaluate three systems:

- **System 1**: Acetic acid production (1% CO_2 conversion).

- **System 2**: Methane production (1% CO_2 conversion).

- **System 3:** Methane production (99% CO_2 conversion).

However, System 3 was excluded due to significant technical and economic limitations, as detailed in Deliverable 5.2: Sustainability Assessment of the Two Hybrid Systems.

The TOPSIS method was implemented under 1000 weighting combinations. Figure 3.1 b) shows that System 1 (acetic acid production) achieves significantly more favourable outcomes (green points) compared to System 2 (methane production), even under worst-case scenarios.

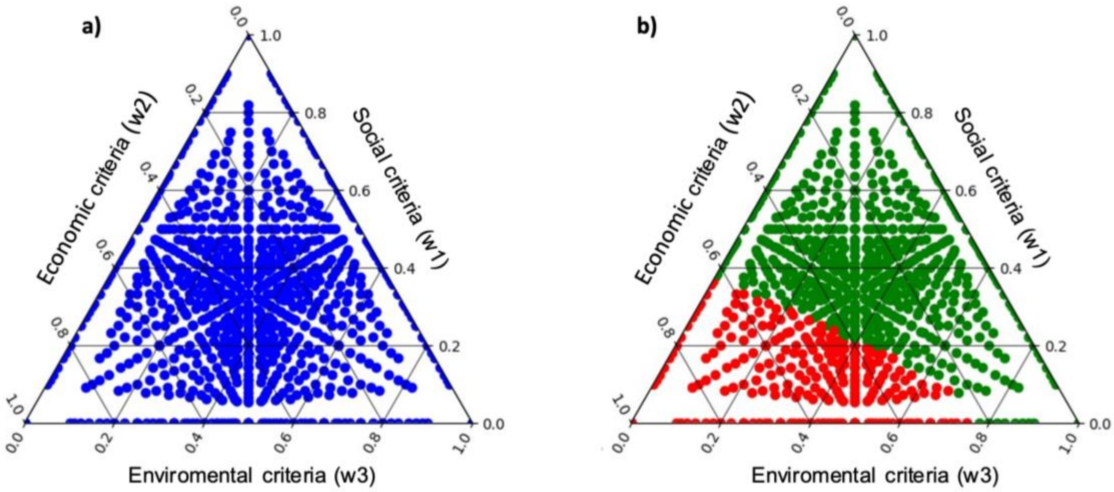

*Figure 3.1. TOPSIS results for the studied systems: System 1 (Acetic Acid) with 1 % CO_2 conversion (Green dots), System 2 (Methane) with 1% CO_2 conversion (Red dots), System 3 (Methane) with 99% CO_2 (Blue dots). Being **w1 = Social criteria**, **w2 = Economic criteria** and **w3 = Environmental criteria**. **a)** Best case scenario, **b)** Worst case scenario.*

System 1 demonstrates strong **technical and economic** advantages:

- It uses *Moorella thermoacetica*, a well-studied and stable microorganism, ensuring process robustness.
- The production cost of acetic acid is \$0.32/kg, far below the fossil-based alternative (\$1.02/kg), while System 2's methane production remains uncompetitive at \$22.9/kg.

In contrast, System 2 faces technical challenges, including low CO_2 conversion efficiency, which increases energy demand for downstream processes such as methane separation and purification.

Regarding the **sustainability assessment**, the MDO analysis and triangular comparisons confirm that System 1 outperforms System 2 across environmental, economic, and social criteria:

- Environmental: System 1 achieves a carbon-negative footprint of - 0.78 kg CO_2-eq/kg, surpassing System 2's higher carbon intensity.
- Social: System 1 supports EU-based production, aligning with higher labour standards and social responsibility goals.

In conclusion, System 1 (acetic acid production) is the optimal solution for upscaling due to its technical simplicity, economic feasibility, and environmental sustainability, offering a scalable, cost-effective, and robust alternative to fossil-derived production systems.

4. CONCLUSION – SELECTED DEVICE FOR UPSCALING

After a comprehensive analysis and comparison of the methane and acetic acid production systems, it is evident that **the acetic acid system, utilising M. thermoacetica, presents a clear advantage over the methane system** based on several key factors. These factors include higher efficiency, scalability, and a more simplified reaction pathway.

The comparison between both systems highlights the following advantages of the acetic acid production system:

- Efficiency: The methane production system achieved very low yields, with a result of 0.22% Rel Area % CH_4 from gas chromatography, whereas the acetic acid system demonstrated significantly higher yields (detailed in D2.2), indicating greater overall efficiency. Additionally, TZE's experiments showed that POZ-M NPs exhibited the highest efficiency for electron transfer, enhancing metabolic activity and acetic acid production, which improves the overall performance and scalability of the system.
- Scalability: The methane system's reliance on Cdots as photosensitizers poses significant challenges for scaling, making it less viable for industrial applications. In contrast, the acetic acid system exhibited more robust and scalable characteristics, which are crucial for large-scale implementation.
- Simplified Pathway: The direct carbon fixation pathway in the acetic acid system, utilising the Wood-Ljungdahl pathway, eliminates intermediate steps such as hydrogen generation, leading to reduced complexity and lower energy losses. This makes the system more efficient and easier to scale.
- Metabolic Stability: The growth and metabolic stability of M. thermoacetica under thermophilic conditions offer a higher level of adaptability to industrial-scale processes, making it a more reliable option for long-term operations.

In terms of sustainability, the MDO methodology applied in this study, which incorporates environmental, economic, and social criteria, further supports the superiority of the acetic acid system. The TOPSIS analysis revealed that:

- Acetic Acid Production achieved significantly more favourable outcomes, even under worst-case scenarios, outperforming the methane systems in both technical and economic aspects.
- **Environmental Sustainability:** The acetic acid system demonstrated a carbon-negative footprint of **-0.78 kg CO_2-eq/kg**, surpassing the methane system's higher carbon intensity, making it the more environmentally friendly option.
- **Economic Feasibility:** The production cost of acetic acid in is $0.32/kg, which is substantially lower than the fossil-based alternative ($1.02/kg) and the uncompetitive cost of methane production at $22.9/kg. This makes acetic acid production not only more sustainable but also far more cost-effective.
- **Social Responsibility:** Acetic acid system aligns with higher labour standards and social responsibility goals, supporting EU-based production and contributing to sustainable economic growth.

In conclusion, acetic acid production emerges as the optimal choice for upscaling due to its technical simplicity, robust scalability, economic competitiveness, and significant environmental benefits.

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