



**Proof of concept shown for operating a dual
chamber membrane reactor for the production of
PHACOS**

Deliverable D4.1

31 January 2023

Fabián Moreno Avitia, Álvaro Gómez Luengo, Juan Nogales Enrique

CNB-CSIC

PROMICON

**Harnessing the power of nature through PROductive Microbial
CONsortia in biotechnology - measure, model, master**



Prepared under contract from the European Commission

Grant agreement No. 101000733
EU Horizon 2020 Research and Innovation action

Project acronym: **PROMICON**
Project full title: **Harnessing the power of nature through PROductive Microbial CONSortia in biotechnology - measure, model, master**

Start of the project: June 2021
Duration: 48 months
Project coordinator: Dr. rer. nat. Jens O. Krömer
Helmholtz Centre for Environmental Research - UFZ
<https://www.ufz.de/>

Deliverable title: Proof of concept shown for operating a dual chamber membrane reactor for the production of PHACOS

Deliverable n°: D4.1
Nature of the deliverable: Demonstrator
Dissemination level: Public

WP responsible: WP1
Lead beneficiary: CSIC

Citation: Moreno-Avitia F., Gómez-Luengo A. & Nogales, J. (2023). *Proof of concept shown for operating a dual chamber membrane reactor for the production of PHACOS*. Deliverable D4.1 EU Horizon 2020 PROMICON Project, Grant agreement No 101000733.

Due date of deliverable: Month n° 24
Actual submission date: Month n° 24

The content of this deliverable does not necessarily reflect the official opinions of the European Commission or other institutions of the European Union.

Table of contents

1. Summary	4
2. Design	4
1. Ceramic chamber selection	4
2. Dual-chamber photobioreactor setup	4
3. Operation	7

1. Summary

The objective of Task 4.1 is to develop a dual-chamber photobioreactor for microbiome-based bioprocesses. Unlike conventional single-strain culture, multi-strain-based bioreactors require addressing unique design and operation features. Therefore, we have developed a dual-chamber photobioreactor to allow the co-cultivation of two or more different strains exploring the division of labor concept at the level of strain and space.

2. Design

1. Ceramic chamber selection

In order to achieve the desired performance of the dual-chamber bioreactor, it was mandatory to analyze the diffusion properties of different materials. In the context of this project, we evaluated a span of ceramics mixes with distinct mineral compositions. These ceramics were provided by our collaborators from LIQUIFER Systems Group GmbH. The mineral content is not the only relevant parameter that modifies the evaluated properties. The firing temperature changes the ceramic porous size, density, and strength. A code name was established for each material based on its color and firing temperature. The ceramic R1050 displayed the best result for sucrose diffusion.

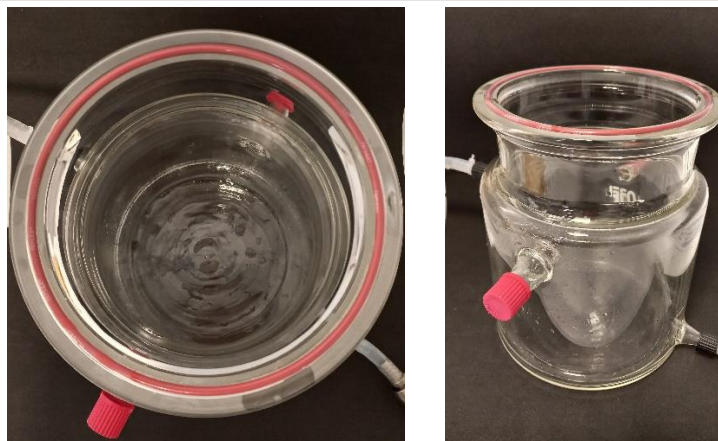
2. Dual-chamber photobioreactor setup

Once the best-performing ceramic material was selected, the final internal chamber for the 2 L photobioreactor was constructed. The dimensions of this ceramic are 10 cm width and 16.5 cm high. This chamber has a total volume of 1300 cm³ and 800 cm³ of operation volume.



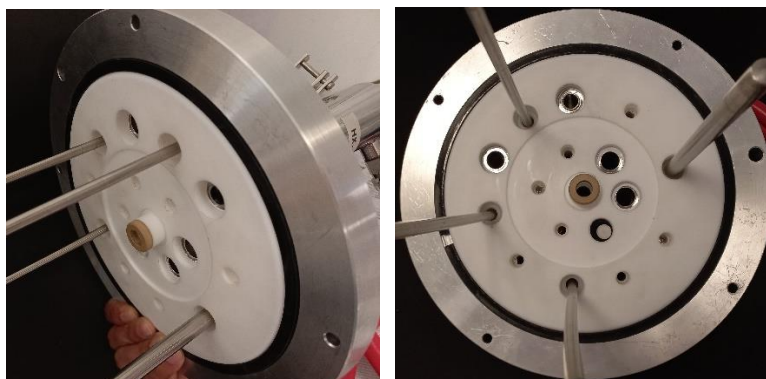
Ceramic chamber 1050.

The external jar was designed with a conservative configuration using standard glass lab-scale bioreactors as a reference. This jar is coated with a water-based heat exchanger system that does not block the light incidence over the microbial culture. Light transmission is a critical parameter for the farming module.

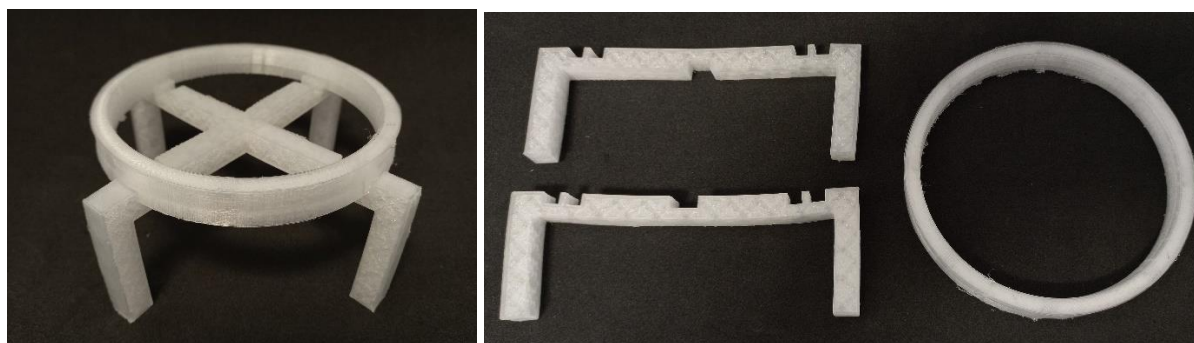


Crystal photobioreactor jar. In the figure, the configuration of the coated jar with its water input and output for heat exchange can be observed.

To guarantee the stability of the inner chamber, the bioreactor lid was modified with a 3 mm deep indentation. This indentation fits with the ceramic chamber and avoids its movement. In the same context, a support was designed to keep the inner ceramic at a distance of 5 cm from the bioreactor bottom. This distance is needed to allow agitation of the external chamber using a magnetic stirrer. Three pieces were designed and 3D printed in polypropylene to form the ceramic chamber platform.



Bioreactor lid. The lid of polytetrafluoroethylene has a 3 mm indentation that fits the inner chamber diameter. Each chamber possesses its own sensors and pipe inputs.



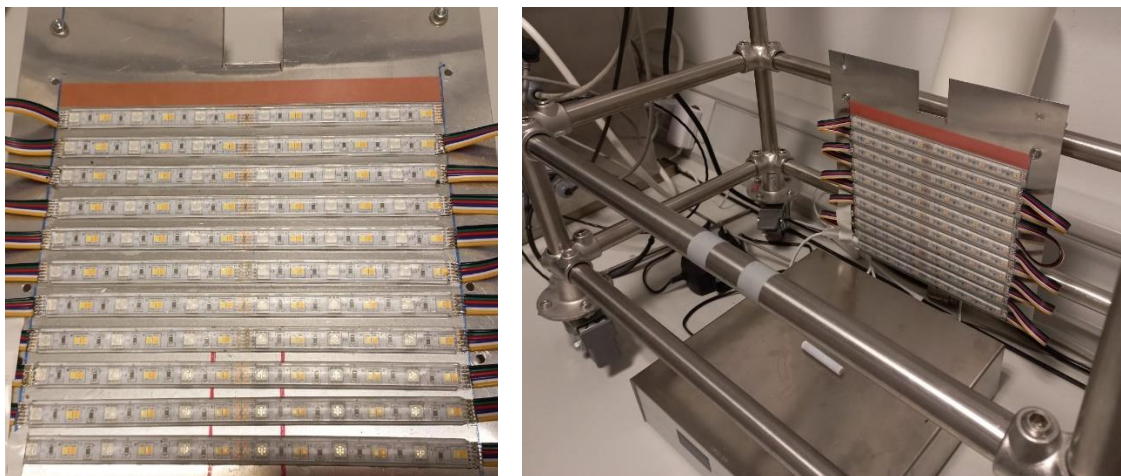
Polypropylene 3D printed stand for the ceramic chamber.

LED display

A personalized led display carries the light supply. The light intensity can be regulated with manual control. This led display was designed to be mounted in the bioreactor rack to reduce

D4.1: Proof of concept shown for operating a dual chamber membrane reactor for the production of PHACOS

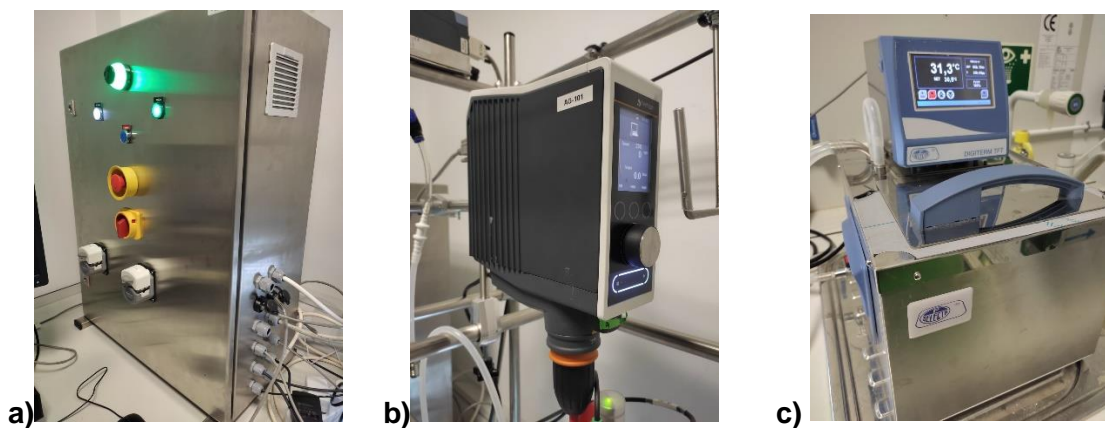
the distance between the light source and culture. Additionally, the light color can also be adjusted using alternative mixes of RGB colors, cold white, and warm white.



LED display. In the left picture, we can observe a close-up of the led display. The right image shows the led panel in its final position.

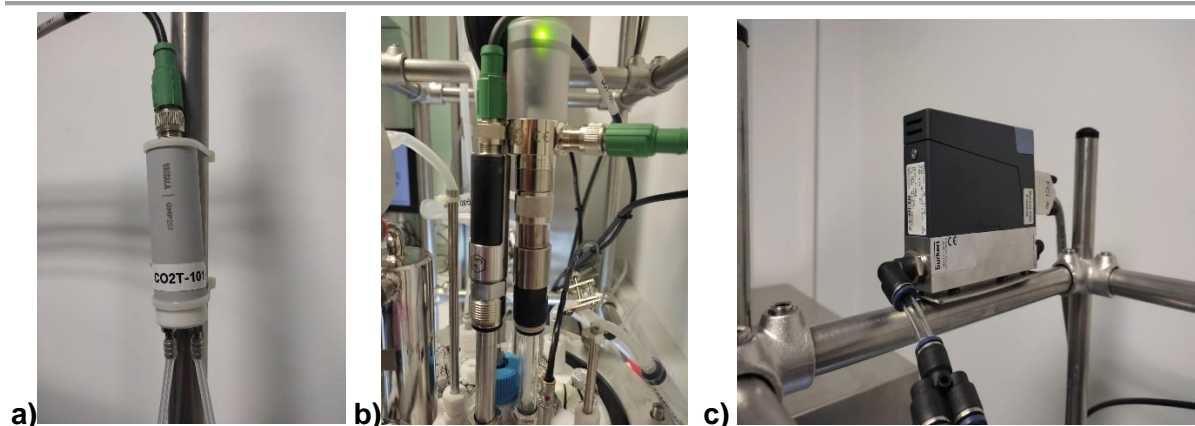
Standard bioreactor components

This bioreactor accounts with standard components for air flow measurement, air and CO₂ input pipes, temperature control, condensation unit, CO₂ and oxygen sensor, and magnetic and paddle stirrers. These devices are shown in the figures below.



Bioreactor devices 1. a) Photobioreactor control tower, b) Impeler motor controller, c) Water heater/chiller.

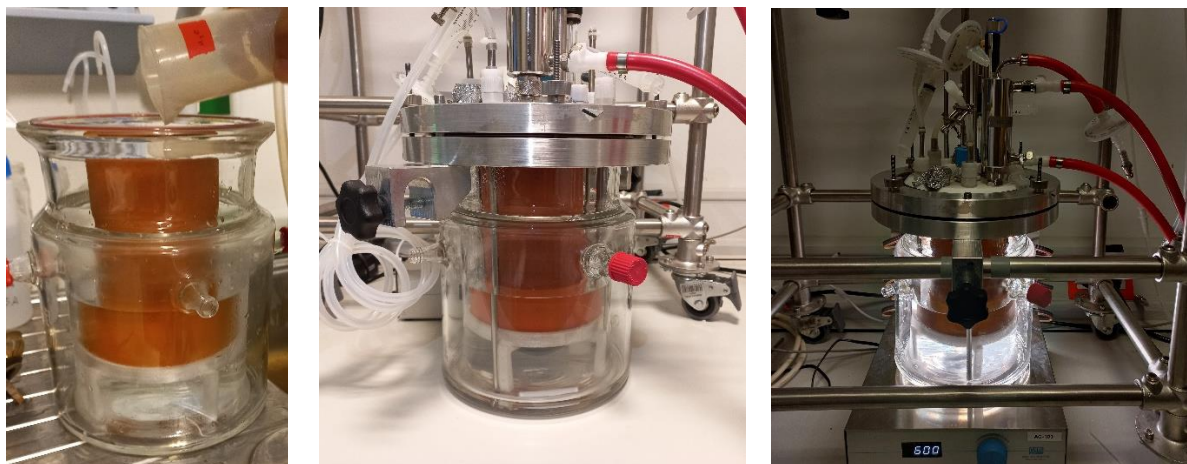
D4.1: Proof of concept shown for operating a dual chamber membrane reactor for the production of PHACOS



Bioreactor devices 2. a) CO₂ air output sensor, b) Dissolved oxygen and pH sensor, c) Air mass flow control.

3. Operation

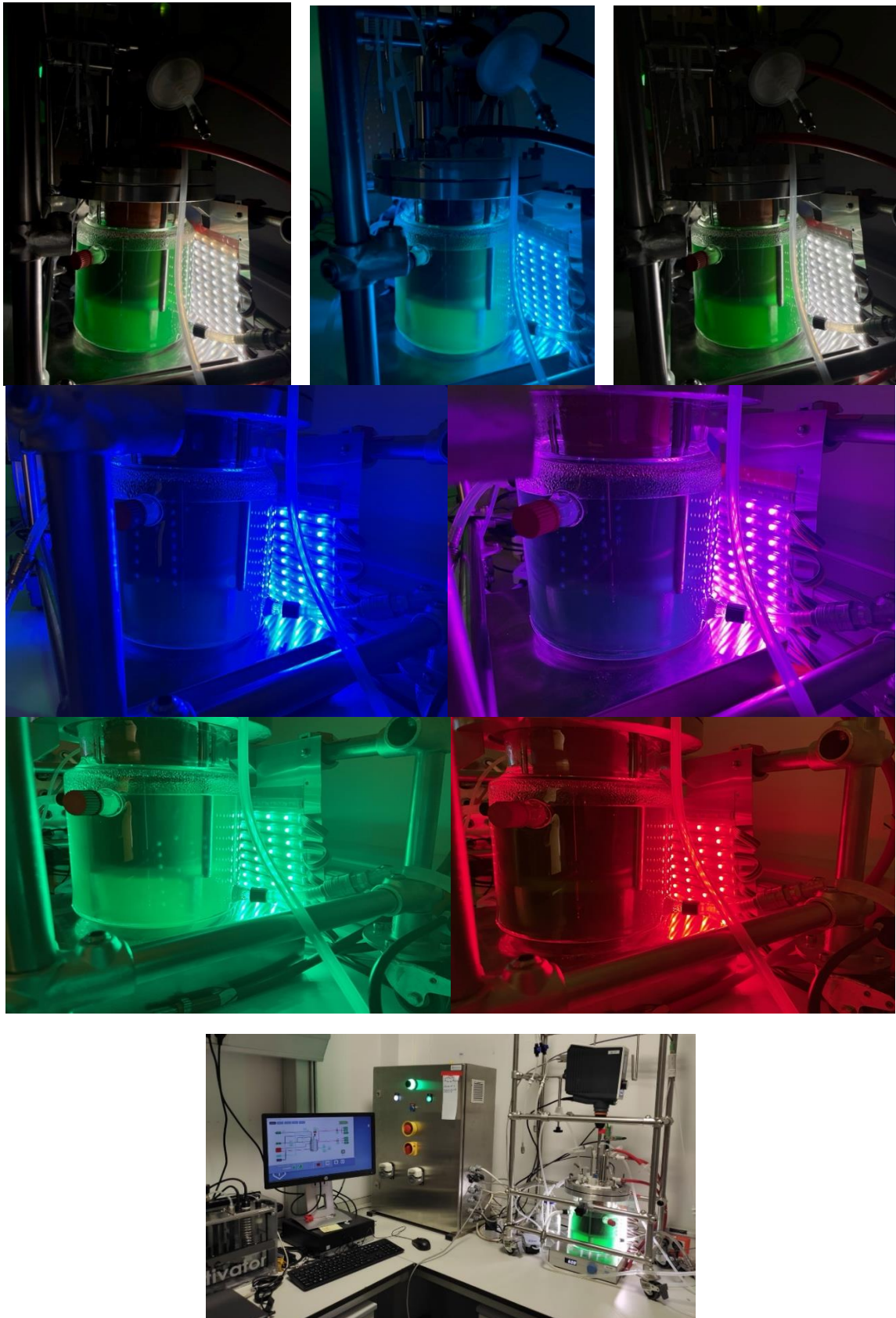
This section will show illustrative pictures of the complete dual-chamber photobioreactor setup.



Complete setup. Each chamber has to be filled individually with the desired culture media. Then, the lid has to be set carefully, avoiding touching the ceramic and sensor. Once the devices are calibrated and set, the culture can be started.

The following images show the operation alternatives related to the light source. The light source can vary the wavelength mix to optimize autotrophic organism culture and more complex light-induced systems.

D4.1: Proof of concept shown for operating a dual chamber membrane reactor for the production of PHACOS



A short video of the performance of the whole two-chamber bioreactor can be found at:

<https://github.com/SBGLab/PROMICON/tree/main/DELIVERABLE.4.1>