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Photofuel - Biocatalytic solar fuels for sustainable mobility in Europe

Deliverable D3.2

Key process parameters for growth and excreted fuel production in a continuous 1 L chemostat

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Editorial		
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Publishable Summary

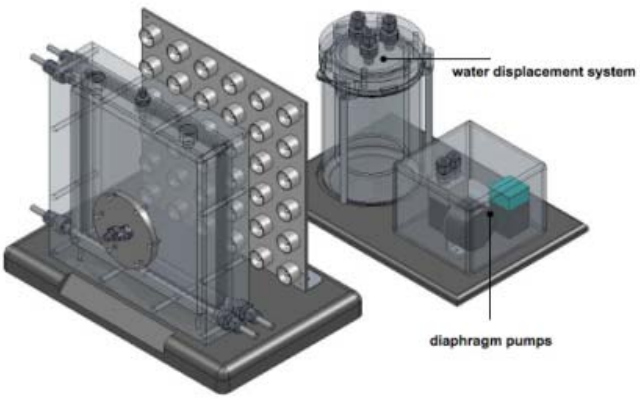
Work Context and Objectives

A continuous production of secreted algae product is envisioned for enabling stable productivity of the fuel product while prolonging cell growth. This would require scale-up and environomics analysis of promising organisms and mutants. The kinetic evaluations of the growth and production rates of viable strains will be then used for establishing a chemostat process with continuous product removal. The objectives of this work are:

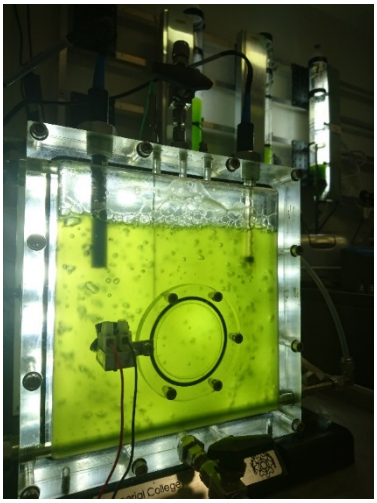
- Identifying cost effective and efficient separation systems to remove excreted fuel components from culture broth, reduce harvesting costs and energy demand by adaptation of the photobioreactor system to the direct production of fuel compounds
- Developing continuous manufacturing and processing operations in a chemostat

Work Performed and Key Findings

The activities carried out throughout this reporting period are:



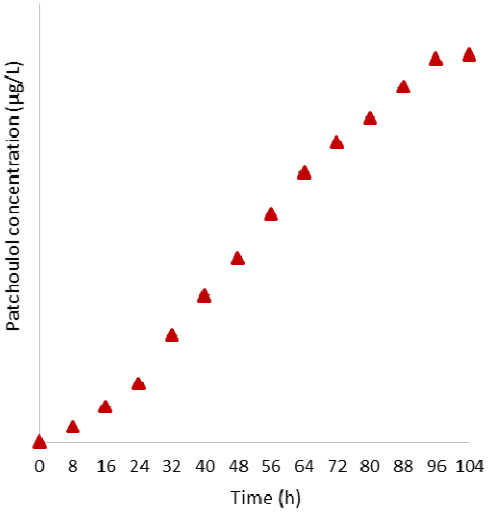
- Construction and operation of a PBR system designed to accommodate production of different secreted products by various algae strains
- Batch experiments on patchoulol, bisabolene and butanol strains forwarded by WP2 which involves culture scale-up from flasks to photobioreactor (PBR), environomics study and analysis of kinetic data
- Chemostat operation conducted to extend culture viability and develop separation systems for continuous removal of secreted products

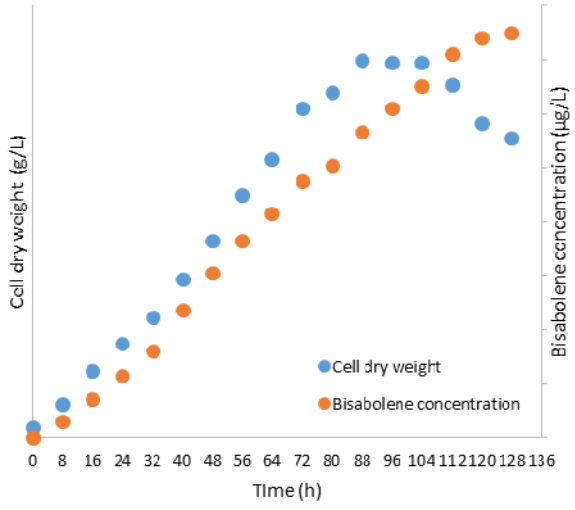


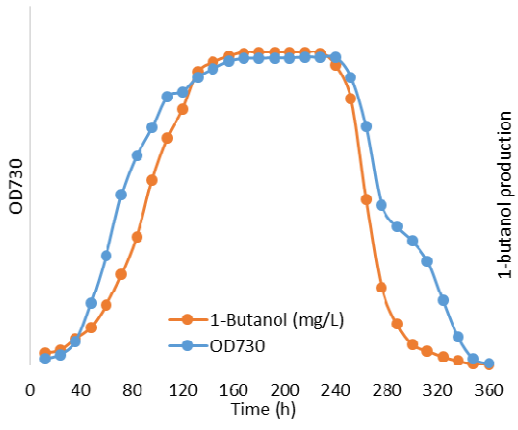
The PBR system development was a key step in achieving the

objectives of the project as various strains with different product lines can be tested. As the process parameters can be fully controlled using the PBR system, the environomics study can be successfully conducted to generate enough kinetic data required to develop the chemostat operation. The separation systems were also developed with the end-product in mind and are compatible with the PBR system; insoluble secreted products can be separated using extraction techniques while soluble products are recovered through pervaporation.

In batch operation of the forwarded strains, three strains producing patchoulol, bisabolene and 1-butanol were tested. The patchoulol strain was studied in a preliminary work where a modified medium was developed and tested. In flask cultures, the patchoulol strain exhibited a growth rate of 0.0063 h^{-1} and maximum biomass concentration of 0.563 g L^{-1} while the bisabolene culture offered a growth rate of 0.0051 h^{-1} and maximum biomass concentration of 0.5633 g L^{-1}. Meanwhile, the 1-butanol strain managed to grow at a rate of 0.211 h^{-1} and maximum biomass concentration of 4.283 (OD_{730}). The algae strains were then cultivated in the flat plate PBRs. The bisabolene strain had grown at a rate of 0.0597 h^{-1} with maximum biomass concentration of 0.803 g L^{-1} while the patchoulol strain achieved a growth rate of 0.0213 h^{-1} and maximum biomass concentration of 1.34 g L^{-1}. The 1-butanol culture had also grown faster at 0.35 h^{-1} and achieved maximum biomass concentration of 4.3 (OD_{730}).







The next task was to evaluate the effects of light intensity, temperature and pH on the growth, nutrient uptake and product excretion. It was found that the best process parameters for patchoulol production comprise a light intensity of 100 µE s-1 m-2, temperature 32°C, pH of 8 and continuous light whereas for bisabolene production, the process parameters were continuous light at 100 µE s-1 m-2, temperature 30°C and pH of 7.5.

Subsequently, 1L batch reactors were modified to operate as chemostats and the continuous excretion of fuel product was evaluated. For the bisabolene culture, the continuous 'milking' of the *C. reinhardtii* culture was demonstrated successfully for the chemostat process in a duration of 10 days. The bisabolene productivity (rate not given for confidentiality reason) was maintained over more than 15 days of chemostat operation. The culture continues to be healthy and the chemostat experiment is still in operation. The 1-butanol strain had also been operated as a chemostat and maintained 1-butanol, too. These findings indicate the potential of continuous product removal by a chemostat flow process for prolonged cell culture and extended production of the secreted products in both cases.