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Photofuel - Biocatalytic solar fuels for sustainable mobility in Europe

Deliverable D3.8

Optimisation of performance of the best wild type algal strains and of *Botryococcus braunii* cultured at pilot level

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Publishable Summary

Outdoor scaling up to a pilot reactor (1400 L, 30 m^2 of occupied land, vertical, east-west oriented) was performed for the two wild type strains of the selected cyanobacteria on which to focus the Photofuel efforts. These strains, *Synechocystis* PCC 6803 and *Synechococcus* PCC 7002, were scaled up from 7-L bubble tubes to the 1400-L vertical GWP®-II reactor through an intermediate step in 40-L GWP®-III reactors (45°inclined, facing south). The pilot reactor was built in a way that different widths of the culture chamber, and thus different volumes, could be selected according the needs of the cultures to be grown. The volume can be varied from 900 to 5000 L. For the trials with Photofuel cyanobacterial strains a volume of 1400 L, corresponding to a width of 4.5 cm, was selected to increase light availability to the cultures, thus increasing volumetric productivity and reducing energy consumption per unit biomass produced. *Synechocystis* PCC 6803 grew well in the first two steps of the scaling-up process and was successfully transferred in the pilot reactor at half capacity, where it showed a lower productivity than in the inclined 40-L reactors. When the culture was diluted to full optimal reactor volume (1400 L), a contamination by the phagotrophic alga *Poterioochromonas* led to culture crash. *Synechococcus* PCC 7002 showed a much higher productivity in the inclined reactor facing south than in the vertical east-west oriented pilot reactor. This strain, which had already proved to be very sensitive to low temperatures, confirmed that the longer time needed by the culture in the pilot reactor to reach an optimal temperature strongly affected productivity. Contamination by the phagotrophic alga occurred also this time, without leading to culture crash, thanks to the marine medium used to grow the strain, but in the long run it affected the quality of the culture. The eurihalinity characteristics of *Synechocystis* PCC 6803 and the sensitivity to salinity of the phagotroph were used to try to limit the effect of the contaminant of the culture. The culture did not crash but, also in this case, its quality was strongly affected.

The two reference strains, *Nannochloropsis oceanica* F&M-M24 and *Botryococcus braunii* Showa, were also scaled-up to the pilot reactor.

Nannochloropsis oceanica F&M-M24 was grown in nitrogen sufficient medium for inoculum production until the volume of 700 L achieved in the pilot GWP®-II reactor. Once a sufficient concentration was reached, the culture was diluted to the final optimal reactor volume (1400 L) using nitrogen-deprived medium and the nitrogen starvation phase for lipid induction started. The 4.5 cm culture width (corresponding to 1400 L volume) was chosen instead of 15 cm (corresponding to 5000 L volume) to provide a higher amount of light to the cells, thus favouring the lipid accumulation process. The productivities obtained in the different steps of the scaling-up process were in line with those expected from literature. The lipids were about 60% of the dry biomass at the end of the starvation phase and total fatty acids about 50%. The fatty acid profile showed as usual an increase in saturated fatty acids (C14:0 and, mainly, C16:0) and monounsaturated fatty acids (C16:1 and C18:1)

Botryococcus braunii Showa confirmed to be a challenging strain for mass cultivation. Cultures of this strain were initially performed indoors in a 60-L annular column to obtain the inoculum to start outdoor scale-up. This culture was used to inoculate 40-L GWP®-III reactors and these, in turn to inoculate the 1400-L GWP®-II reactor. Due to the low productivity and very long times to produce sufficient inoculum, it was not possible to grow this alga at a width higher than 4.5 cm (1400 L volume), as this alga would have probably preferred due to its light sensitivity. To avoid excess radiation, the cultures were thus shadowed in the early phases of growth. The culture obtained in 40-L GWP®-III reactors was also used to inoculate two 1-m^2 raceway ponds, one operated under nutrient replete conditions and one under nitrogen starvation. Besides low productivity and sensitivity to variations in many environmental factors, contamination represented the main problem. However, hydrocarbon contents up to about 30% of dry biomass were attained.