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Photofuel - Biocatalytic solar fuels for sustainable mobility in Europe

Deliverable 3.1

Evaluation of performance of wild type algal strains under laboratory conditions in prospect of the scaling-up steps

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Publishable Summary

The scope of the Photofuel project is to obtain biofuels from a direct to fuel approach using GM photosynthetic microorganisms (microalgae and cyanobacteria). The aim of the experimentation at UNIFI is to provide information on wild type strains as close as possible to the strains used for genetic manipulation. The final aim is to evaluate the strains outdoors in photobioreactors suitable for scaling-up and thus providing information on ~~cultivation~~ parameters that will be useful in large-scale cultivation of the modified strains.

During the first stage of the project the wild type strains were tested at laboratory scale. The microalga on which the work was focussed is *Chlamydomonas reinhardtii* (both with and without cell wall) while the cyanobacteria were *Synechocystis* and *Synechococcus*. Besides these wild type strains, a reference microalga, *Botryococcus braunii*, the only able to naturally produce and excrete hydrocarbons suitable for fuels, was tested.

All the strains were cultivated in 300-mL or 1-L bubbled tubes under controlled conditions to evaluate the productivity potential adopting light intensities and light:dark periods as close as possible to outdoor conditions. Before the start of the trials to establish the effect of culture parameters on growth, a long work was performed, especially for *C. reinhardtii* to design a culture medium suitable for outdoor cultivation, by changing those components too expensive to be used for commodities production or those components of the medium that need laborious operations to be prepared. The aim was to devise a simple medium, as cheap as possible and easy to be prepared also by non-specialist operators.

From this work it emerged that scaling up a culture using ammonium as nitrogen source is problematic, due to the decrease of pH following ammonium uptake, which finally might halt growth and lead to culture crash. This would also interfere with the supply of carbon as CO_2, and growth could become difficult to control. For this reason, other *C. reinhardtii* strains able to grow on nitrate were tested, resulting that when pH stability is attained no significant growth difference were present with strains growing on ammonium or on nitrate, except for the nitrate-growing wall-less mutant, which was the worst performer.

Synechocystis and *Synechoccocus* wild type strains showed a similar productivity than *C. reinhardtii* when cultivated in the same culture system. For *Syenchococcus*, trials to choose a culture medium easier to prepare were also performed.

The two *B. braunii* strains tested, belonging to race A, were slow to adapt to bubbled culture systems and after adaptation the productivities were low in one strain, that was abandoned. The other strain gave interesting productivities, especially when grown at higher light intensity. Hydrocarbon production was observed in both strains but it was not quantified. In the more promising strain, which will be taken outdoors in the following experimental phase, hydrocarbon production was higher under high light. This is a positive feature in view of outdoor cultivation.

Culture parameters such as biomass concentration at start, pH and salinity most suitable to attain the highest productivity were evaluated, so as to have information useful for transferring the cultures outdoors.

This laboratory work allowed to select the best strains for outdoor cultivation, to be performed in the flat plate photobioreactor Green Wall Panel®, a patent pending design of Fotosintetica & Microbiologica S.r.l., spin-off the University of Florence.