

## Microalgae for biofuels: the Portuguese experience

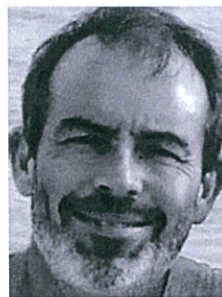
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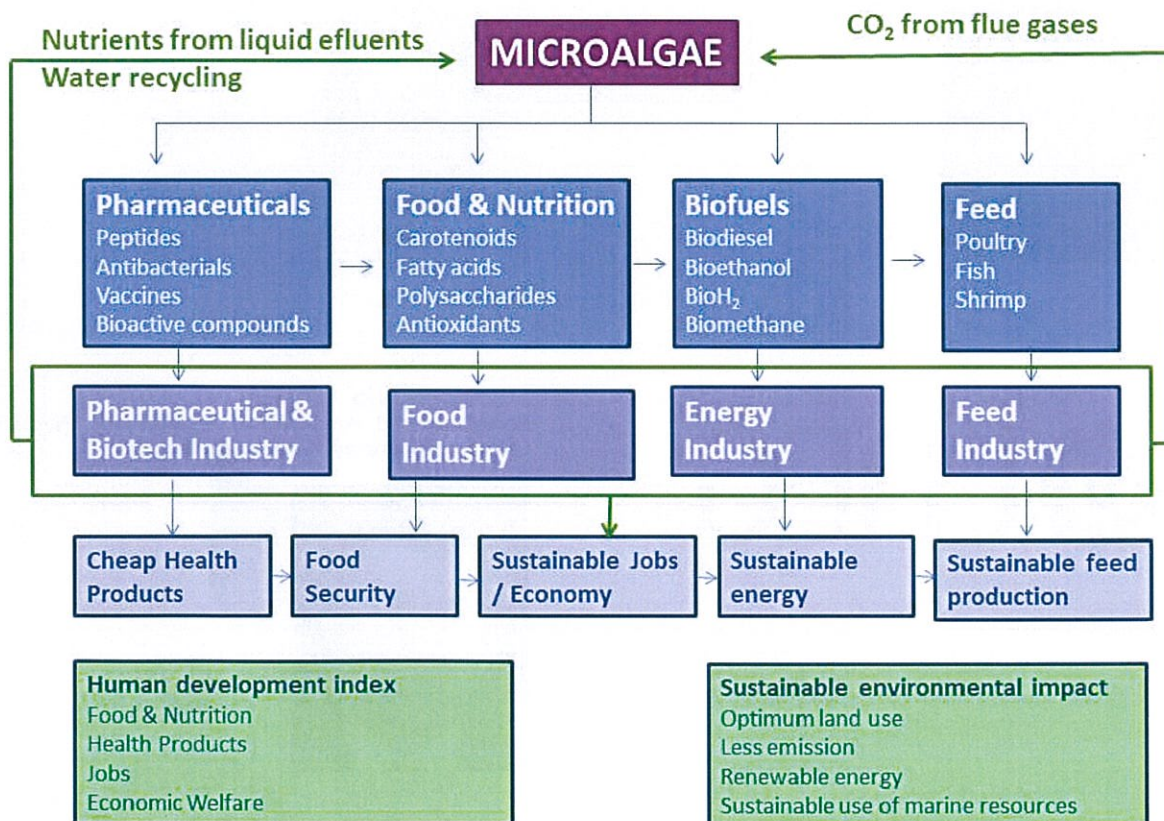


Autotrophic microalgae are photosynthetic organisms that undergo the conversion of light into chemical energy as a form of a wide range of organic compounds through its photosynthetic machinery. The cultivation of microalgae brings environmental advantages, bearing in mind the capability of nutrient recycling in wastewaters together with the fixation of greenhouse gases such as CO<sub>2</sub>.

These micro-organisms have been widely recognized as having huge potential as feedstock for food, feed, pharmaceutical and cosmetic industries (carotenoids, antioxidants, polyunsaturated fatty acids, single-cell proteins (SCP), phycobiliproteins, polysaccharides, vitamins, phytosterols, minerals). Microalgae have also been proposed as a feedstock for bioplastics, agriculture biofertilizers and recently as an energetic vector towards the production of a wide range of biofuels. Microalgae exhibit clear advantages when compared with higher plants, such as higher photosynthetic efficiency, higher aerial biomass productivities, higher CO<sub>2</sub> biofixation rates (many polluting focus such as cement and thermoelectric plants can be used), higher O<sub>2</sub> production rates, non-competition for agricultural areas (marginal lands such as deserts, rocky areas and salt pans can be used), non-competition for drinking waters (saltwater, brackish water and wastewaters can be used), harvesting routines can be carried out daily with a better equipment and resources management trimming storage costs. Several constraints should be overcome in order to achieve a cost-effective microalgal biofuel production, such as high energy inputs and still prohibitive production costs (currently around 5000 €/ton, far above the desired threshold target of 700 €/ton).

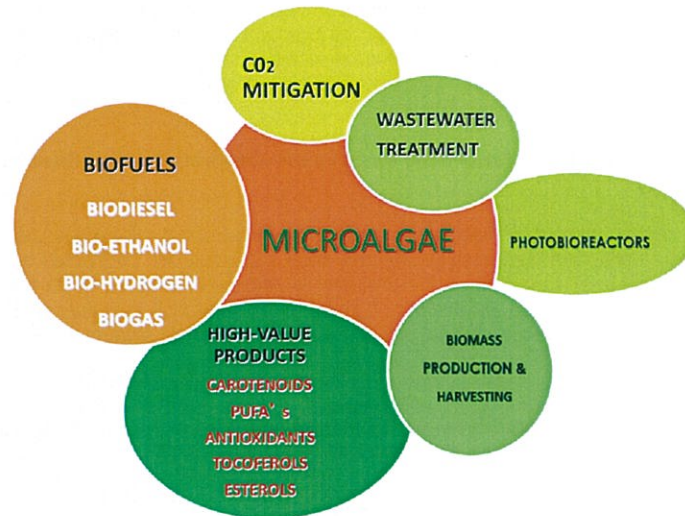
In order to become reality R&D investment should be preceded in the improvement of biomass productivity and harvesting together with cell wall breakage and adequate and efficient metabolite extraction. These topics have been widely covered by the Bioenergy Unit within the National laboratory for the Energy and Geology (LNEG) in Lisbon, Portugal (formerly the Renewable Energy Department within the INETI- Nacional Institute for The Engineering, Technology and Innovation). Its microalgal biotechnology research group has been the pioneering group in microalgal biotechnology in Portugal with more than 25 years of experience and a national and international well known reputation (Fig 1).

Figure 1  
Microalgae biorefinery



Source: adapted from Subhadra (2010)

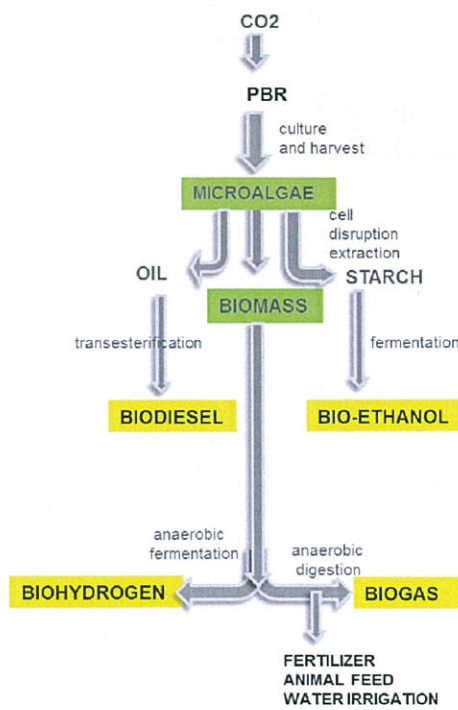
Figure 2  
R&D areas at Bioenergy Unit – LNEG- National Laboratory of Energy and Geology (Portugal)



The Bioenergy Unit has been developed research in the above referred areas (Fig 2) through some emblematic National and International Projects, such as:

**Microalgae as a sustainable raw material for biofuels production (Fig 3)**

Figure 3  
Microalgae as a sustainable raw material for biofuels production (biodiesel, bioethanol, bio-H<sub>2</sub> and biogas)  
(PTDC/ PTDC/AAC-AMB/100354/2008)



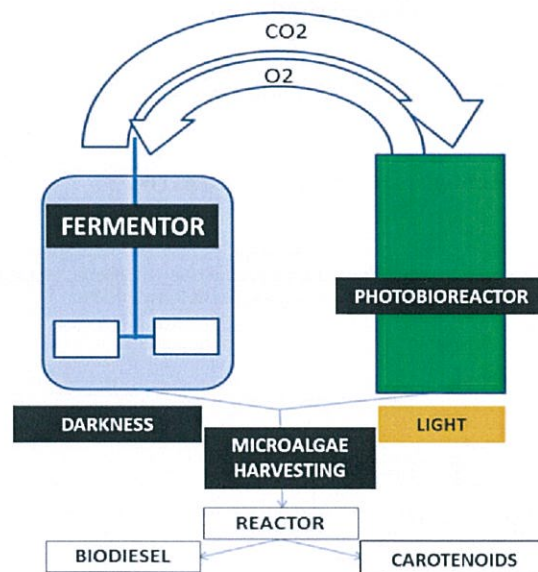
Source: <http://www.lneg.pt/iedt/proyectos/392/>

The project addressed the optimization of some innovative technologies for harvesting, dewatering, cell disruption and extraction of valuable metabolites and conversion of the microalgal biomass into biofuels. The integrated valorization of the all energy vectors (biodiesel, bioethanol, biohydrogen and biogas), using the biorefinery concept, were highlighted on the project. Life Cycle inventory, technical and economical evaluation was also performed to a quite a few value chains. Several publications came out from this project. Some concerning the production of a single biofuel (e.g. biodiesel (Gouveia and Oliveira, 2009), bioethanol (Miranda et al., 2012), biohydrogen (Marques et al., 2011; Baptista et al, 2014) and other related biorefineries for the production of biodiesel, pigments and biohydrogen (e.g. Nobre et al., 2013).

#### Symbioalga- New symbiotic approach for a truly sustainable integrated microalgae production directed to a biorefinery platform (Fig 4)

The target of the project was to produce biodiesel from auto and heterotrophic microalgae using an innovative symbiotic approach. Two reactors were used, one photoautotrophic and other heterotrophic, connected by the gas phase. The outlet gas from the auto one (O<sub>2</sub> enriched) was used as the inlet gas for the heterotrophic. In parallel, the outlet gas from the hetero one (CO<sub>2</sub> enriched) was used for the autotrophic. This symbiotic approach increases biomass and oil productivities, greater than the sum of the productivities of the two bioreactors operating separately, thus diminishing operational production costs (Santos et al., 2011).

Figure 4  
**SIMBIOALGA - New symbiotic approach for a truly sustainable integrated microalgae production directed to a biorefinery platform (FCOMP-01-0124-FEDER-013935)**

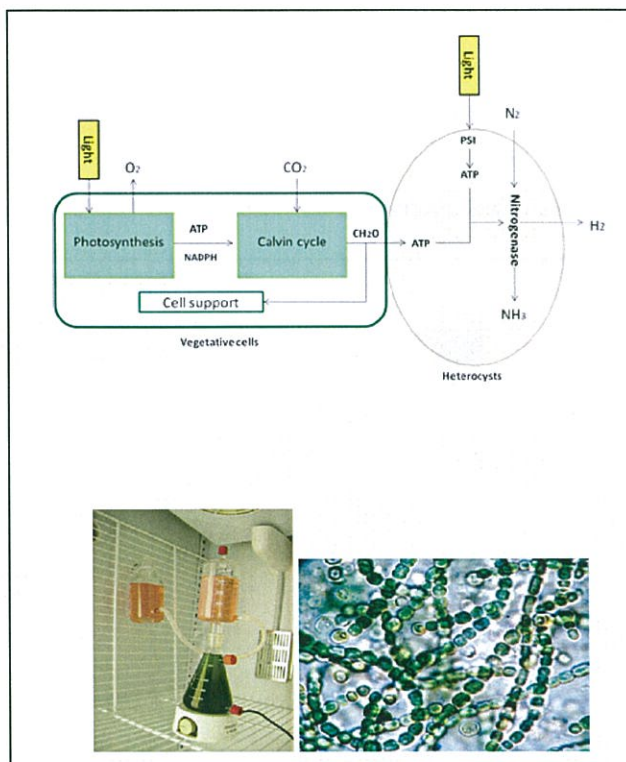


Source: <http://www.lneg.pt/iedt/projectos/393/>

#### Biohydrogen production from the cyanobacteria *Anabaena* sp. and its mutants (Fig 5 a, b, c)

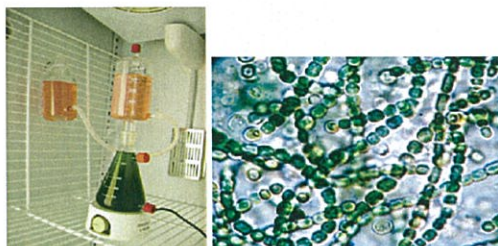
In this project the production of H<sub>2</sub> was done by growing a nitrogen fixing cyanobacteria *Anabaena* PCC 7120 wt and its mutants (deficient in uptake and/or bidirectional hydrogenase enzymes), using mineral media under light and aerobic conditions. Evaluation of H<sub>2</sub> production was performed by testing and optimizing cultivation parameters. A separation and purification process of hydrogen-rich gases was developed using a hot Pd-based hydrogen gas separation system integrated in the bioreactor (Marques et al., 2011).

Figure 5  
 Biohydrogen production from the cyanobacteria *Anabaena sp.* and its mutants. PTDC/ENR/68457/2006



Vegetative and heterocyst cells metabolism showing  $H_2$  production (Picture on the left)

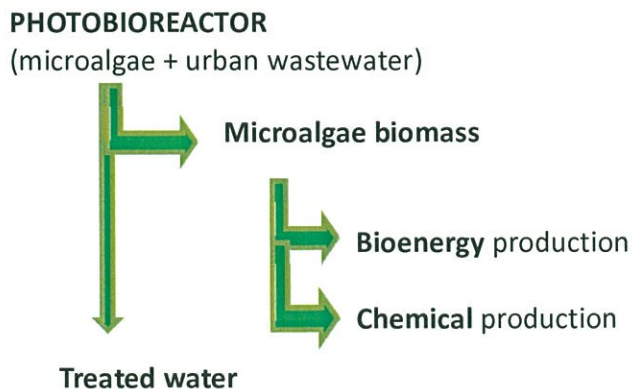
$H_2$  produced collection system (Picture on the right)



*Anabaena sp.* Strain (Picture on the left)

ww-SIP - From Wastewater Treatment Plants to Biorefineries (Fig 6)

Figure 6  
 From Wastewater Treatment Plants to Biorefineries (LIFE+)

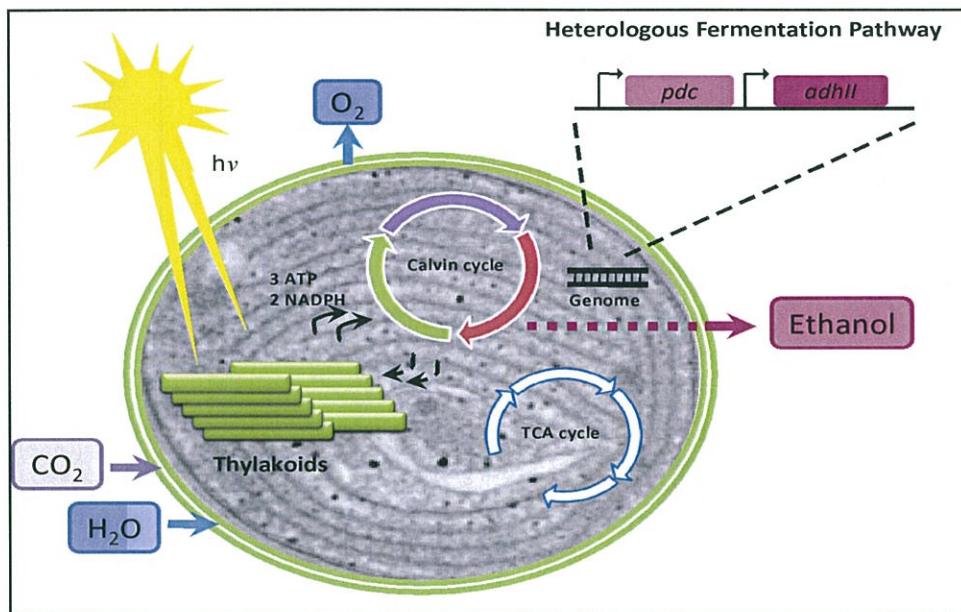


Source: <http://www.lifewwsip.it>

This project aims the simultaneous treatment of urban wastewater using microalgae and the energetic valorization of the obtained biomass. The nutrient removals were quite high and the treated water fits the legislation (PT Dec-Lei 236/98) in what concerns the parameters analyzed (N, P, COD). The obtained biomass was converted into biohydrogen (bioH<sub>2</sub>), a clean energy carrier, through dark fermentation by the bacteria *Enterobacter aerogenes* (Batista et al., 2015). This is an ongoing project.

### DEMA - Direct Ethanol from microalgae (Fig 7)

Figure 7  
DEMA- Direct Ethanol from microalgae(FP7). Cellular metabolism for ethanol drop-in production

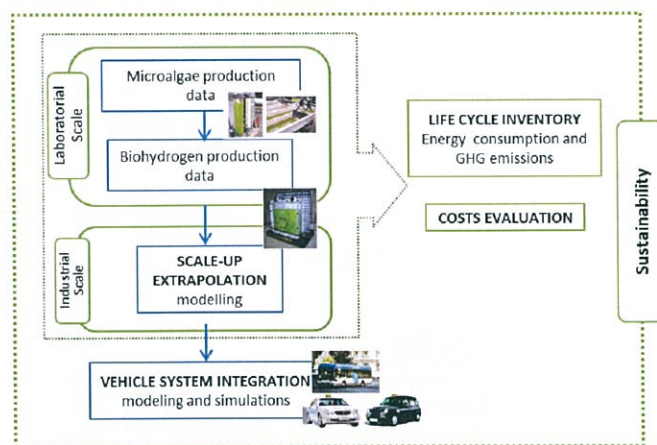


Source: <http://www.dema-etoH.eu>

The goal of the project is to develop, evidencing and licensing a complete and economically competitive technology for the direct production of bioethanol from microalgae, with low-cost scalable photobioreactors. The initial proof-of-concept results show via Life Cycle Assessments (LCA) and economic balance that using microalgae to produce bioethanol for less than € 0.40/L is feasible. The catalytic conversion of solar energy, H<sub>2</sub>O and CO<sub>2</sub> into ethanol is been carried out by a metabolically engineered strain of the cyanobacterium, *Synechocystis* sp. PCC 6803. This project still proceeds.

**ESIBITS - Evaluation of the Sustainability of Industrial Biohydrogen production from microalgae, and Integration in taxi/bus Transport Systems (Fig 8)**

Figure 8  
ESIBITS – Evaluation of the Sustainability of Industrial Biohydrogen production from microalgae, and Integration in taxi/bus Transport Systems EXPL/EMS-ENE/1078/2012

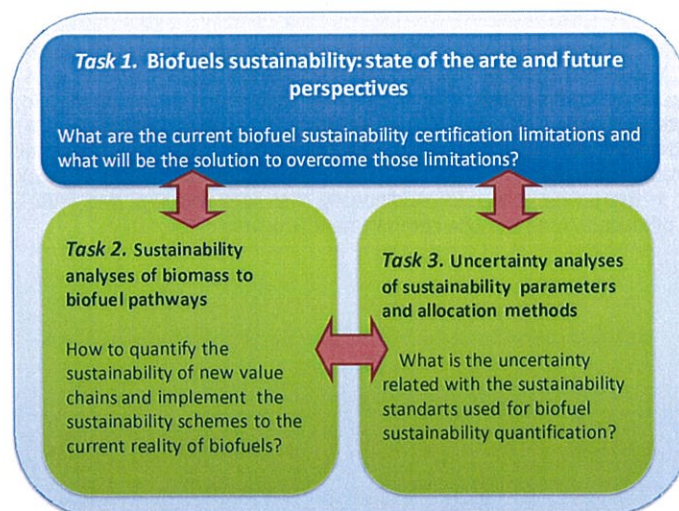


The present project aimed to the evaluation of the potential of biohydrogen production at industrial level, from microalgae, with a possible application to the road transport sector, namely taxi and bus fleets.

The whole process regarding the culture of microalgae and extraction of oil, pigments and biohydrogen was taken into account for energy and emission evaluation and life cycle co-product credits. Aiming a future implementation of biohydrogen for use in road transportation, the scale-up is highly relevant to determine the potential of competitiveness of the biohydrogen fuel comparatively to conventional fossil fuels when used in specific road vehicle fleets. Present and future estimations for economic viability taking into account the hydrogen fueled vehicle penetration in the taxi/bus fleet market were considered (Ferreira et al., 2013).

**BioSustain - Sustainable mobility: Perspectives for the future of biofuel production (Fig 9)**

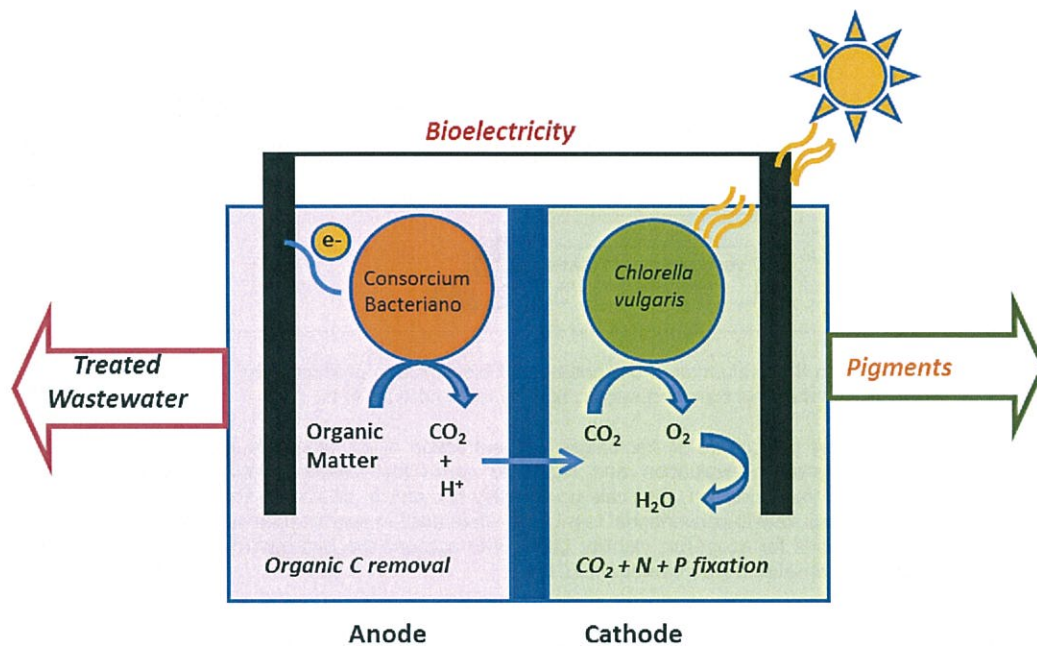
Figure 9  
BioSustain - Sustainable mobility: Perspectives for the future of biofuel production.  
PTDC/EMS-ENE/1839/2012



The objective of this project is to develop comprehensive life-cycle models to characterize the environmental, energetic and economic performance of advanced biofuels value chains (in particular from microalgae and lignocellulosic materials). Furthermore, the project aims to create new perspectives for future biofuels, especially by: identifying the more sustainable technologies available and incorporating uncertainty in biofuel life-cycle sustainability assessment (LCA), in order to increase reliability of the results. This project is on road.

### PMFC – Photosynthetic Microbial Fuel Cells (Fig 10)

Figure 10  
Photosynthetic Microbial Fuel Cell (*Chlorella vulgaris* microalga in the cathode electrode)

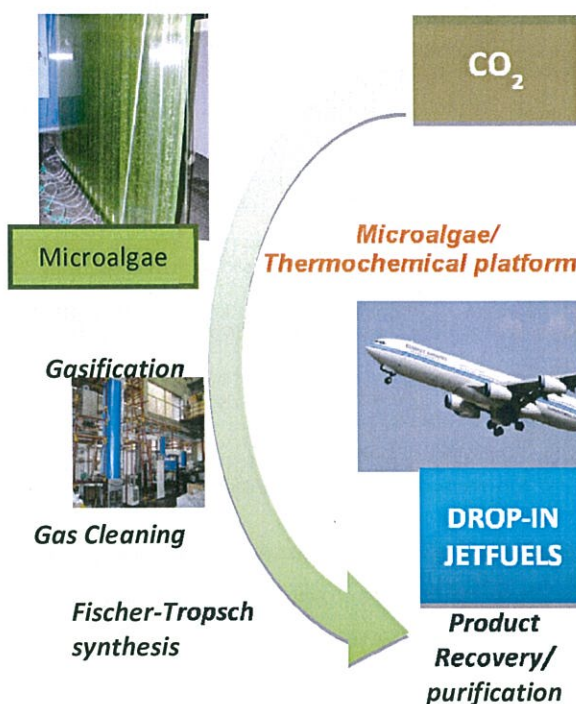


Source: adapted from Gouveia et al. (2014)

This study demonstrates the simultaneous production of bioelectricity and added-value pigments in a Photosynthetic Alga Microbial Fuel Cell (PAMFC), with a concomitant wastewater treatment and carbon fixation. A PAMFC was operated using *Chlorella vulgaris* in the cathode compartment and a bacterial consortium in the anode. In this compartment bacteria reduced the carbon source and produced protons and electrons that were transported to the cathode compartment, producing electricity. In the cathode the microalga in the presence of carbon dioxide/bicarbonate and light produced oxygen, reacts with the protons and electrons producing water. The results shown that increasing light intensity leads to an increase of about 6-folds in the power produced. Additionally, the light intensity and PAMFC operation potentiated the pigment carotenogenesis, produced in the cathode compartment (Gouveia et al., 2014).

## Advanced fuels for Aviation using Thermochemical platform of Microalgae (Fig 11)

Figure 11  
Advanced fuels for Aviation using Thermochemical platform of Microalgae



The evaluation of the potential of microalgae to jet fuel production is the target of the project. The thermochemical platform, through gasification and Fischer-Tropsch will be envisaged.

### Conclusion

Microalgae have been widely recognized as a key value chain for the bioeconomy. The European SET-PLAN (strategic energy technological plan) through the EBTP (European Biofuels Technological Platform), the EIBI (European Industrial Bioenergy Initiative) and the EERA Bioenergy (European Energy research Alliance) recognizes the strategic importance of micro- and macro-algae as a key value chain for advanced and other biomaterials production. Moreover, past and current set of R&D project Calls in the frame of the H2020 and COST Actions have also been evidencing the importance of microalgae in this context.

On the other hand, Portugal as a coastal European country with excellent edafoclimatic conditions, has a great potential for microalgae culturing in a sustainable way, helping in food/feed, wastewater and flue gas treatment, as well as in biofuel supply security. Besides fisheries and aquaculture, microalgae farms (similarly to agriculture farms) could contribute for the blue growth/blue economy and could have an especial importance on the development of the coastal communities. However, this activity it is not only restricted to the coast as fresh water and brackish water microalgae can be easily grown inland, providing economical and societal advantages in several depressed rural areas. These farms could be important source of nutrition, employment and income.

Portugal is also the second largest fish consumer per capita, thus a quality and quantity of microalgae biomass produced is also essential for fish farming, as microalgae is the basis of the food chain. Portugal has several spots with more than 3000h/year insolation hours, together with adequate temperature profile along the year, constitute some of the most remarkable competitive advantages.

Other nearby regions with similar edafoclimatic conditions, such as the Mediterranean basin, will strongly benefit from the Portuguese experience. The close cooperation between these countries under the frame of research programs and human mobility will be very welcome in the near future, and is imperative to accelerate this promising field of R&D.