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Experimental plant for the cultivation of microalgae in photobioreactors for energy production

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Abstract: The continuous research of alternative and sustainable energy solutions with respect to fuels deriving from oil has led the current industrial and scientific system to analyze and develop approaches and technologies capable of enhancing materials of different nature for the production of biofuels. Algae are an alternative widely studied for this purpose, not only respect to the production of fossil fuels, but also respect to first-generation biofuels that use higher plants produced by normal cultivation methods.

There are many plant solutions tested and disseminated internationally, operating in both outdoor and indoor environments. One of the most widespread criticisms is the inability to supply biodiesel quantities suitable for a production scale and with a positive economic and energy balance.

This paper describes the results of a 4-years experimental research project oriented to the development of a low-scale demonstration plant of a complete advanced energy system based on the massive cultivation of microalgae and their treatment aimed at competitive production and sustainable bio-oil and biogas having requisites of suitability and compatibility with the relevant reference markets. The article intends to focus on a specific technological macro-component, completely studied and realized during the research project: the transparent, fully closed tubular photobioreactor (PBR) made of plastic material operating in continuous and in outdoor environments used for algal cultivation at low cost and high efficiency.

The experimental plant was developed with the aim of providing a contribution to the main critical situations spread in this field, in particular: the need to reduce costs of the components of the plant and of the input resources necessary for the functioning of the system (energy, fertilizers, CO_2, water, etc), as well as to maximize its modularity, reproducibility and exportability in other territorial contexts.

Keywords: algae cultivation, photobioreactor, bio-oil from microalgae

1. Introduction

At the international level, the evolution of energy scenarios and sustainable development strategies have favoured a substantial transformation of the energy system based on a more secure, sustainable and low-carbon economy [JRC, 2015]. In this scenario, biofuels play a significant role in order to reduce oil dependency and increase the sustainability of the energy sector [Gerbens-Leenes P. et al., 2014]. Biofuels had a technological diversification, starting from the first products deriving from crops of an oleaginous nature, to those derived from wood-cellulosic materials and finally arriving at biofuels produced from algal-type crops. This evolution is articulated in three generations of biofuels, each with very different performances. The use of first generation biofuels has generated a lot of controversy, due to their impact on global food markets and on food security. The IEA data underline that, in 2011, 1% of the world's available arable land is used for the production of biofuels, providing 1% of global transport fuels [IEA, 2011]. This has pushed research towards third-generation biofuels, using in particular algae as raw material. Algae refer to a highly diverse group of eukaryotic organisms (about 100,000 species), mostly containing chlorophyll. Based on their size, algae can be classified into two major groups: macroalgae (from 50 cm up to 60 m) and microalgae (from nano- to milli-meters) [Darzins A. et al., 2010].

Their use as biomass useful for the production of biofuels starts from the first experiences in Japan, US and Germany since the 1950s, especially towards the use of microalgae [Brennan L. and P. Owende, 2010]. Indeed, thanks to their morphological, structural and chemical features, the biofuels yields of microalgae are much greater than those of macroalgae [Chen H. et al., 2015]. Microalgae are generally more efficient converters of solar radiation, water, CO_2 and nutrients into usable energy via photosynthesis. Microalgae have generation times that are usually higher than 24h (sometimes less than 8h) and an efficiency of the photosynthetic process of about 5%, but can present higher peaks [Lam M.K. and Lee K.T., 2012; McKendry P., 2002]. Considering the potential benefits, numerous private or public funded projects are supporting algae (both micro- and macroalgae) production and research. A study conducted by the IEA [IEA, 2017] has surveyed over 400 internationally active research projects, most of which are concentrated in Europe.

Through specific treatment extraction processes, this biomass is the primary source for the production of different types of fuel. Figure 1 summarizes the possible algal biomass conversion processes for biofuels production. Thanks to the lipid content, microalgae have high potential for biodiesel and biogas production. The processes for the production of bioethanol and hydrogen are less investigated [Martín M. and Grossmann I.E., 2014].

Tipically, conversion pathways for fuel production refer to the cultivation and processing of algae and include harvesting and some form of cell pre-treatment to prepare the algal biomass for extraction of intracellular lipids, in combination with the recovery and purification of other products [IEA, 2017]. Each step takes place through sub-systems which, when properly combined, make up the complete system. These systems can be schematized as shown in Figure 2, where AD means "Anaerobic Digestion", DAF means "Dissolved Air Flotation", GHG means "Greenhouse Gases", HTL means "Hydrothermal Liquefaction" and SLS means "Solid Liquid Separation".

The cultivation phase will be the focus of this study. Cultivation can use systems classified as: open or closed. The first are in direct contact with the atmosphere and adopt configurations as circular or raceway pond. The closed ones include the isolation of the reagent system from the external environment and can be classified as tubolar reactor; flat plate reactor; pyramid reactor; fermenter type reactor; hybrid reactor [Placzek M., 2017]. Cultivation systems can also be classified according to their location in indoor (with the need for artificial light sources) or in an outdoor environment. Unlike open pond, closed reactors guarantee mono-species algal culture for very long times and a simple control system for nutrients, growth, temperature, CO_2 and pH, resulting in higher productivity (considering the volume of the system). However, these have a high initial cost and are very specific for the cultivated microalgae strain [Chisti Y., 2007].

The development of the market linked to microalgae still encounters many obstacles. Effective algal cultivation for biofuels production requires a combination of technical breakthroughs including cultivation parameters under different locations-specific conditions [IEA, 2017]. Moreover, algae cost more per unit mass than other second-generation biofuel crops due to high capital and operating costs. The main barrier to the production of biofuels from algae [Alabi A.O. et al., 2009; Van Iersel S., 2009; Lundquistl T.J. et al., 2010] are: significant initial capital investments; the pre-commercial state of technology development; planning of economic viability and of the plant requires very specific knowledge; higher likelihood of social and environmental impacts for large-scale facilities.

The activities related to the implementation of the technological system of cultivation, extraction and processing of microalgae have considerable costs that represent an important limiting factor, as schematically reported by Spruijt J. Et al. (2014). In the examples reported by the authors, the investments for the part of cultivation and harvesting have a similar cost for open pond plants, while it is about 4 times greater the cost of the part of cultivation in the cases of tubular PBR and PBR flat panels. This suggests that the search for technical solutions and low-cost materials may contribute to making PBR more appealing to the market. This is also a conclusion presented by Torzillo and Zittelli (2015).

Finally, an important global current obstacle is also the decline in petroleum prices since August 2014 which has discouraged investments in this sector linked to the production of biofuels [IEA, 2017].

This article intends to describe the results of a 4-years activity of industrial research and experimental development that has resulted in the development of a low-scale demonstration plant of a complete advanced energy system based on the massive cultivation of microalgae and their treatment aimed at competitive production and sustainable bio-oil and biogas having requisites of suitability and compatibility with the relevant reference markets. Considering the limits of the sector, in order to maximize the yields of the plants destined for algal crops, great attention has been paid to the management of the optimal growth parameters of the algae cultivated, as well as to the optimization of the plants and materials used. In particular, the paper intends to focus on a specific technological macro-component, completely studied and realized during the research project: the transparent, fully closed tubular PBR. Specifically, the results of the research conducted on the plastic materials used for the development of the PBR circuits will be described.

The plant for the integrated production of biofuels and high-added-value compounds from microalgae described in this paper is the main objective of a national research project financed by Italian Ministry of Education, University and Research and allowed to develop a plant solution starting from a TRL 3 to a TRL 5. The prototype plant was developed and tested at Marina di Gioiosa Jonica (RC), identified as a favourable area for the cultivation of microalgae in the outdoor environment thanks to its local characteristics. The activities were dedicated to the study and development of the individual components that constitute the prototype plant, as well as to their experimentation, to the analysis of the efficiency and to the evaluation of the environmental impacts.

2. Plant design

The development of plant solutions capable of supplying biofuel quantities adequate to an industrial scale and with a positive balance from an economic and energy point of view typically encounter considerable limitations from the technical and energy points of view.

The system studied by the ALGENCAL project attempted to overcome some of these critical issues, focusing on the design of technological solutions capable of reducing the use of energy resources, using low-cost materials with a high reproducibility and exportability. Each sub-system that constitutes the overall plant was designed and developed by applying an operational methodological approach composed of the following steps: 1. Analysis of the reference literature and identification of possible available best practices; 2. Analysis of the local reference context (Marina di Gioiosa Jonica); 3. Identification and evaluation of possible plant solutions suitable for the local context; 4. Design, planning, testing, optimization and / or re-design of technologies and operational approaches for each sub-system; 5. Overall assessment of the sub-system in relation to its operation with respect to the plant in its complete configuration; 6. Analysis of impacts.

2.1. Design of the algae cultivation system

In the design and sizing of an algal biomass cultivation system, several variables play a crucial role which significantly affect the behaviour of algae during the growth process: the starting algal strain, the light, the nutrients (including CO_2), the temperature and the operating management methods (agitation, flow rates, contact times and growth). These aspects must be monitored over time and associated with the speed of algae development through growth models and related kinetic equations. These choices are also influenced by plant factors, in particular: the volume of the crop; the temperature; energy consumption; maintenance needs; the quality of the desired final product.

Considering that for the production of high quantities of algal biomass, with quality characteristics not compromised by external factors, the literature suggests the use of PBR that are easy to control and able to guarantee a mono-species algal culture for very long times [Chisti Y., 2007] and taking into account the meteorological and climatic characteristics of the site selected for the experimentation (Marina di Gioiosa Jonica - Italy), the type of reactor selected to be designed and developed in the present project is a Horizontal Tubolar PBR.

In the design of a cultivation system of this type, the necessary plant choices refer to the following aspects: dimensioning and choice of PBR circuit materials, methods of mixing the culture liquid and algal biomass, injection systems of the inoculum and of gases; control systems (temperature, nutrients, gas) and auxiliary services (e.g. cooling and coverage, irrigation, bottom cloth insulating with respect to the ground).

2.2 Study of the materials of the PBR circuits

By definition, the PBR material must be transparent to allow the passage of adequate luminous intensity necessary for algal growth [Wang B. et al., 2012] and, at the same time, able to hermetically isolate microalgae from the external environment. The materials normally used for this purpose are glass, polyethylene (PE), polycarbonate (PC), polyvinyl chloride (PVC), acrylic (Plexiglas, PMMA), silicate and fiberglass [Katuwal S., 2017; Cañedo J.C.G. and Lizárraga G.L.L., 2016; Posten C., 2012]. Table 1 shows the main physical and light characteristics of these materials.

Table 1: Physical properties of PBR construction materials [Katuwal S., 2017]

	Glass	Polyvinyl chloride (PVC)	Polyethylene (PE)	Polycarbonate	Plexiglas, PMMA	Fiber glass
Energy Content (MJ/kg)	25	74	78	-	131	11
Modulus of elasticity (psi)	9137377	420000	530000	-	425000	-
Poisson's	0.20	0.410	-	-	-	-

ratio		@73° F				
Material Density (kg/m3)	2230	1400	920	-	1180	-
Melting point (°C)	-	60	136	-	140	-
Shear strength (psi)	-	-	10500	-	9000	-
Material life span (yrs)	20	-	3	-	20	-
Tensile strength (psi)	-	7450	6240	-	9600	-
Light transmission (%)	-	75	92	-	95	90
Critical Angle	43°	-	46°	-	42.16° -45°	-
Refractive Index	1.52	1.50	1.51	1.60	1.49	-

The choice of the material to be used must be functional with respect to the following properties, strictly connected to the reference context where the PBR must operate: I) mechanical properties of resistance during laying and use; II) chemical-physical transparency and durability; III) bio-compatibility with cultivated algae and chemical compounds added to the culture fluid. Furthermore, aspects linked to the actual availability of the material on the market and its economic compatibility must also be considered. In the preliminary design phase, the plastic materials were preferred to glass thanks to their characteristics of economy, lightness, manageability and limited maintenance requirement, against the risk of less duration compared to rigid materials. However, if appropriately added with suitable UV absorber, antioxidant, photostabilising, etc. additives, plastics can also guarantee remarkable durability (some years).

Among the available plastic materials, the Linear Low-Density Polyethylene (LLDPE) was selected for experimentation. It is an economic material in terms of both purchase costs and management and replacement costs; it is light and thin, manageable and in need of limited maintenance. Moreover, it is a material already commercially available in large sizes, suitable for covering the modules that make up the PBR. From the mechanical and chemical-physical point of view, LLDPE has a high resistance to tearing, impact and puncture, high flexibility and elongation capacity, possibility of use also for making thin films, it has good resistance to chemical agents and air, good electrical properties, good gloss and transparency. In literature there are also several studies that have used this type of material for the realization of PBR [Placzek M. et al , 2017; Huang Q. et al., 2017; Narala R.R. et al., 2016, Harris L. et al., 2013; Wang B. et al., 2012].

The experiments conducted during the project activities involved different samples of LLDPE. In particular: one sample produced specifically for the project thanks to the collaboration of two Italian companies operating in the field of compounding and bubble extrusion of plastic films (sample #1); another developed by adding an additive to sample #1 to make it more resistant to physical and chemical atmospheric phenomena, in particular, anti-UV, HALS (hindered amine light stabilizers) and antioxidant (primary and phospholytic) (sample # 1 + add), two commercial samples produced and marketed by two different Italian companies (samples #2 and #3).

Mechanical resistance. The technical standards U.STR.01, ISO 527-3 and UNI EN 12311-2: 2002 have been applied. The tests were conducted on rectangular test tubes measuring 25x200x0.3 mm. For each tube (diameter 230 mm), the samples were extracted in the "longitudinal" and "transverse" direction, with respect to the tube extrusion direction. In this way, 4 tubes were analyzed for each material (Figures 3): ET (transverse without crease), ETP (transversal with crease), EL (longitudinal without crease) and ELP (longitudinal with crease). Sample #1 was analyzed with and without the addition of additives. 5 measurements were repeated for each test. Altogether, 80 measurements were collected.

Toxicity test according to the UNI EN ISO 8692: 2012 and OECD Guideline n. 201. The test was performed on 20 cm^2 surface samples. 6 replicates per sample are compared with 6 control replicates. After 72 hours of incubation the algal biomass was measured by reading the optical density at 670 nm (Jenway model 6.300 spectrophotometer) in 10 cm optical path cuvettes.

In this study, the results of the toxicity tests were assigned a higher "weight" in the evaluation, compared to the other parameters analyzed. In fact, the other tests describe the properties of the materials which, if necessary, require frequent maintenance interventions. The toxicity test instead represents a property that, if present, prevents the algal strain from growing inside the PBR, making the plant ineffective.

Adhesion test, according to the technical standard ASTM G29-96. The test involves exposing plastic materials to a standardized inoculation of the Oscillatory filamentous alga. Stripes of each plastic material measuring 2.5x6.5 cm. A macroscopic analysis of the materials and an attribution of a numerical value (from 0 to 4) based on the level of algal growth (from 0% to over 60% of the surface coverage) was carried out. A test was performed one time for each sample.

Functional burst tests using compressed air insufflation inside the tubes using a manual tap with a maximum pressure of 10 bar. The tests were conducted with a sequential pressure increase of 0.25 bar. For each sample, 3 replicas were conducted.

3. Results

3.1 Study of the materials of the PBR circuits

3.1.1 Mechanical resistance

In summary, the results of the mechanical tests carried out on the samples are reported in Table 2. The reported values correspond to the average value of the 5 measurements carried out for each test and for each material.

Table 2: Results of mechanical strength tests

Material	ε %	σ Mpa	E Mpa	Note
ET (transverse without crease)				
#1	306	11	100	Necking
#1 + add	542	12	121	Necking
#2	401	11	15	Necking
#3	569	14	110	Necking
ETP (transversal with crease)				
#1	110	10	102	Necking
#1 + add	468	10	119	Necking
#2	360	10	111	Necking
#3	521	13	105	Necking
EL (longitudinal without crease)				
#1	264	13	114	NO Necking
#1 + add	353	14	126	NO Necking
#2	349	14	111	NO Necking
#3	542	15	121	NO Necking
ELP (longitudinal with crease)				
#1	189	13	112	NO Necking
#1 + add	237	13	114	NO Necking
#2	343	14	105	NO Necking
#3	411	13	113	NO Necking

The analyzed samples show an anisotropic behavior with dilatation (ε) more pronounced along the transversal direction than the longitudinal one. The presence of the crease always produces a deterioration in the performance of the material which is drastically reduced for the sample #1. The tension (σ) along the longitudinal direction is greater than the transversal direction. Necking phenomena are observed along the transversal direction. The best properties were found in material #3 which also presents only a weak reduction of ε and σ in the presence of the crease.

3.1.2 Toxicity test

Table 3 shows the average growth rates obtained in the presence of the tested materials and their respective inhibition rates.

Table 3: Toxicity test results

Material	Average growth rate	Inhibition growth rate (%)
#1	1.72	5
#1 + add	0.03	99
#2	1.85	2
#3	1.66	9
Control 1	1.80	
Control 2	1.84	

There is a noticeable difference in algal inhibition rates. The material characterized by greater algal compatibility appears to be #2 with an inhibition value of 2%. The other materials showed higher inhibition values up to 99% observed by exposing the algal test culture to the #1 additive material.

3.1.3 Adhesion test

Table 4 shows the scores assigned to each sample, while Figure 4 shows the image observed at the stereomicroscope (the red arrow indicates the areas of algal growth) on which the evaluation was made. Even if all the samples have excellent performances, three have traces of algal adhesion (#1, #1+add and #2), while one (# 3) is completely free.

Table 4: Results of the adhesion tests

Material	Score	Description
#1	1	Traces of growth (less than 10%)
#1+ add	1	Traces of growth (less than 10%)
#2	1	Traces of growth (less than 10%)
#3	0	No growth

3.1.4 Functional burst tests

Table 5 shows the results obtained for each sample, where similar behaviour is observed for both materials.

Table 5: Results of the burst tests

Material	Test	Breaking pressure (bar)	Note
#1	1	n.a.	n.a.
	2	n.a.	n.a.
	3	n.a.	n.a.
#1 + add	1	n.a.	n.a.
	2	n.a.	n.a.
	3	n.a.	n.a.
#2	1	0.50	Opening hole
	2	1.00	Burst break along the crease
	3	0.75	Opening hole
#3	1	0.75	Burst break along the crease

	2	0.75	Burst break along the crease
	3	1.00	Burst break along the crease

Analyzing the results of the laboratory tests, confirmed also through their experimental use in the field, the most promising materials were #2 and #3. Although #3 reported the best results in mechanical, adhesion and in resistance tests, during toxicity tests it found inhibition values which, although very low, could become dangerous in the mass production process. The sample #2 showed good results in all the tests and the toxicity was absolutely not significant. Therefore the material #2 was the one chosen for the PBR construction.

3.2 Final configuration of the PBR

The final configuration of the implemented PBR is represented by a system composed of six total modules. The first two circuits are used for the growth of the starting inoculum and are respectively 20 m (10 m round and 10 m return) and 200 m long (100 m round and 100 m return) with a maximum volume of 320 l for the first circuit and 3.200 l for the second. The remaining four circuits are dedicated to the biomass production. Each of them is 2 km long (20 tubulars of 100 m each) and occupies a width of 5.60 m (therefore a surface of 560 m^2 in circuit). The tubulars have a diameter of 143 mm, so if completely full, the biomass occupy a volume of 16 l/m of tubular, for a volume of a single section of the tubular (100 m) equal to about 1.600 l. The total volume of the culture in a single 2 km circuit is about 32.000 l (32 m^3), but it must be considered that inside the tubulars a vacuum degree of about 30% is generated, therefore the actual volume of the crop at the interior of the tubes is approximately 22.500 l. The PBR is completed by the inoculum growth tanks and the biomass discharge, the injection pumps, the gas control systems and all the accessory services. Figure 5 shows a photo and a schematic view of the plant.

3.3 Economic considerations

Some summary data about the performance of the plant are shown in Table 6.

Table 6: Some performances of the plant

Indicator	Value
Average biomass concentration	1 g/l
Average yield	10 g/m^2 day
Peak yields	20 g/m^2 day
Annual plant production	25 tons/ha year
Total implementation price	about 15 €/m^2
Dry biomass cost	about 8 €/kg
PBR cost (including labor)	about 13 €/m^2

The PBR designed and developed through the initiative described in this work has a total cost of around 13 €/m^2. This represents a particularly interesting value compared to other studies in the literature (Table 7).

Table 7: Economic assessment from literature review

Study	Cost (€/m2)	Description
Bender A., 2017	51	Vertical Tubular System
Bender A., 2017	80	Flat Panel System
Burns A., 2014	38	Vertical flat panel
Burns A., 2014	50	Flexible film disposable panel
Burns A., 2014	25	Flexible film disposable panel
Burns A., 2014	10	Flexible film disposable panel
Burns A., 2014	15	Flexible film disposable panel
Burns A., 2014	8	Horizontal flexible film panel
Burns A., 2014	24	Horizontal flexible film panel
Burns A., 2014	4	Hybrid trough system
Burns A., 2014	47	Hybrid trough system
Burns A., 2014	50	Hybrid trough system
Posten C., 2012	20	Flat panels
Spruijt J. et al., 2015	286	Tubular PBR
Spruijt J. et al., 2015	303	Flat panel PBR

4. Conclusions

Biofuels produced from algae are among the technologies that have not yet had an appreciable commercial success. Furthermore, these are products with high potential, but with also a high technological risk linked to their performance on a large scale. This represented the objective of the ALGENCAL project, aimed at developing a demonstration plant of a complete advanced energy system based on the massive cultivation of microalgae. This was achieved through an experimental approach aimed at analyzing and developing appropriate solutions capable of optimizing low-cost commercial solutions. The key development issue was to maintain an essential plant layout, using materials and components that are easily available on the market and adopting simple technical solutions, which translate into low plant costs. The experience conducted, in fact, provided important data, useful for the technical assessment of the plant.

The study of the plastic materials used for the construction of the PBR has made it possible to compare different solutions available on the market and to select the one that guaranteed satisfactory performance and safety for the PBR. Compared to all the features analyzed, the toxicological results had a strong impact on the final choice. In fact, although the material #3 was better for all the other tests, its performance on the toxicity test pushed the choice towards material #2 which was used experimentally.

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Appendix A. FIRST APPENDIX

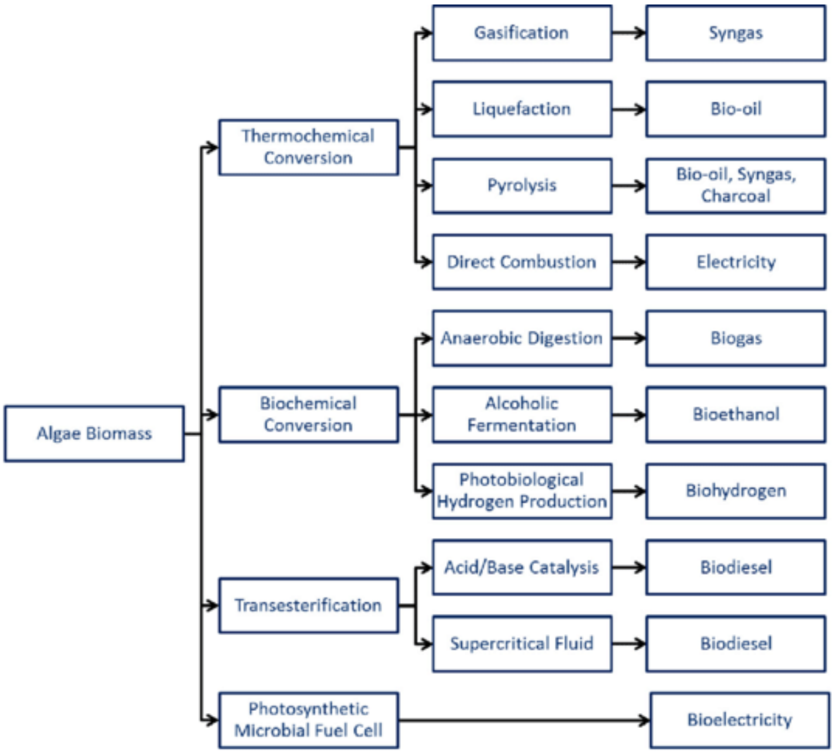

Figure 1: Potential algal biomass conversion processes [Chew K.W. et al., 2017]

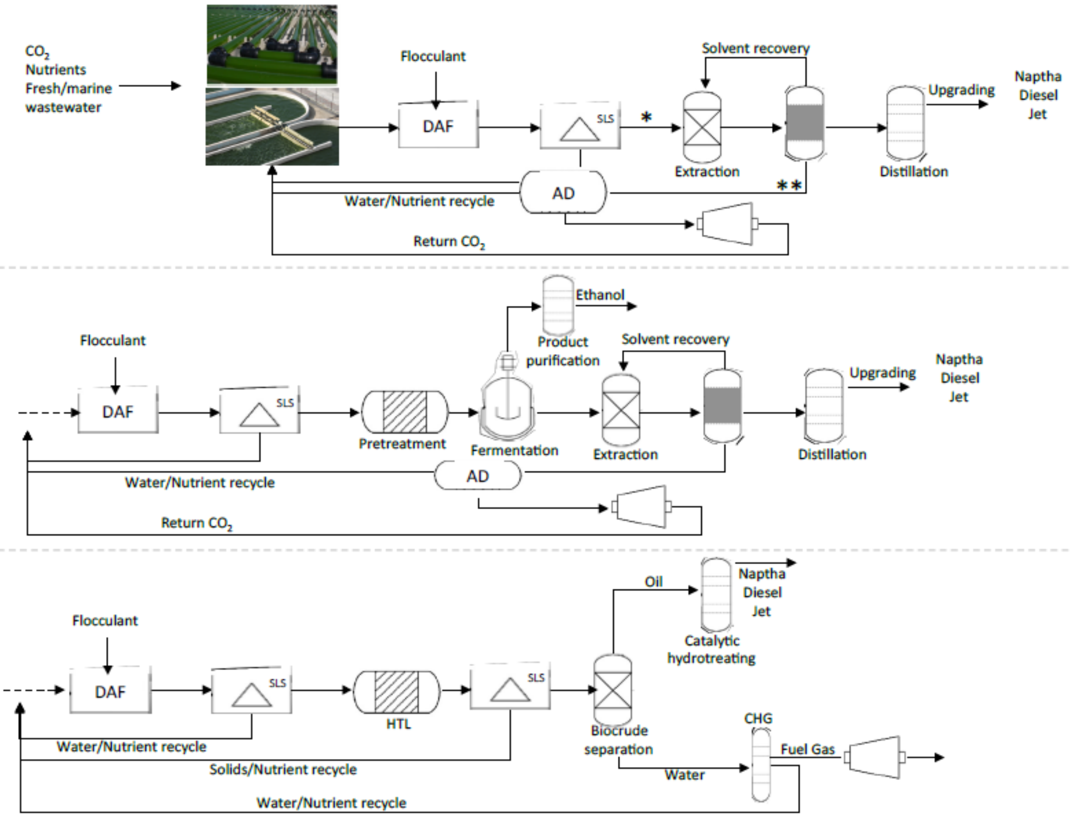

Figure 2: Illustration of major algae conversion pathways under development [IEA, 2017]

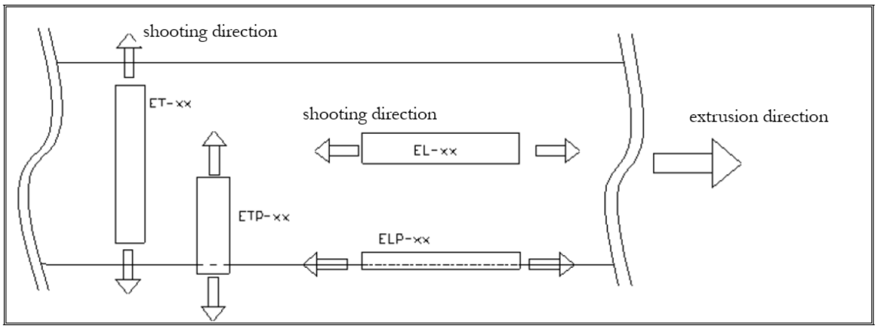

Figure 3: Test samples extraction scheme

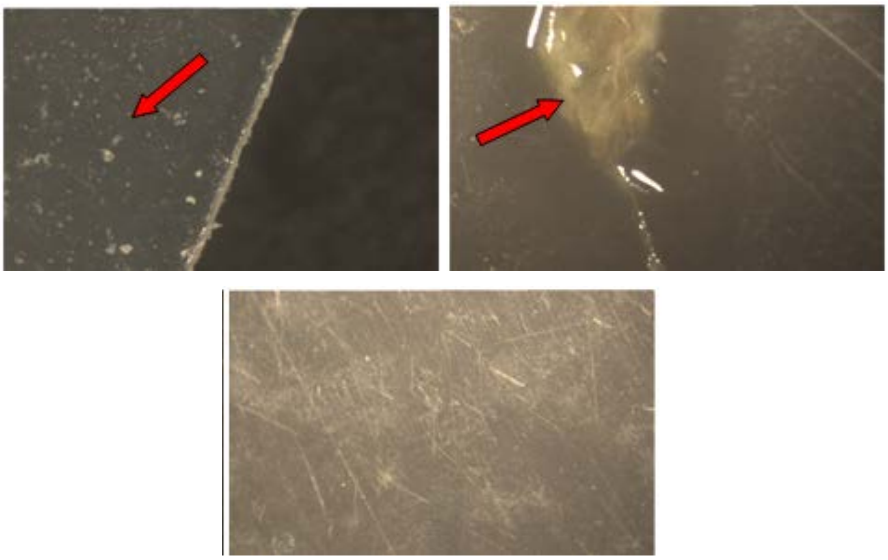

Figure 4: Samples observed at the stereomicroscope (zoom, respectively, at 32x, 25x, 20x)

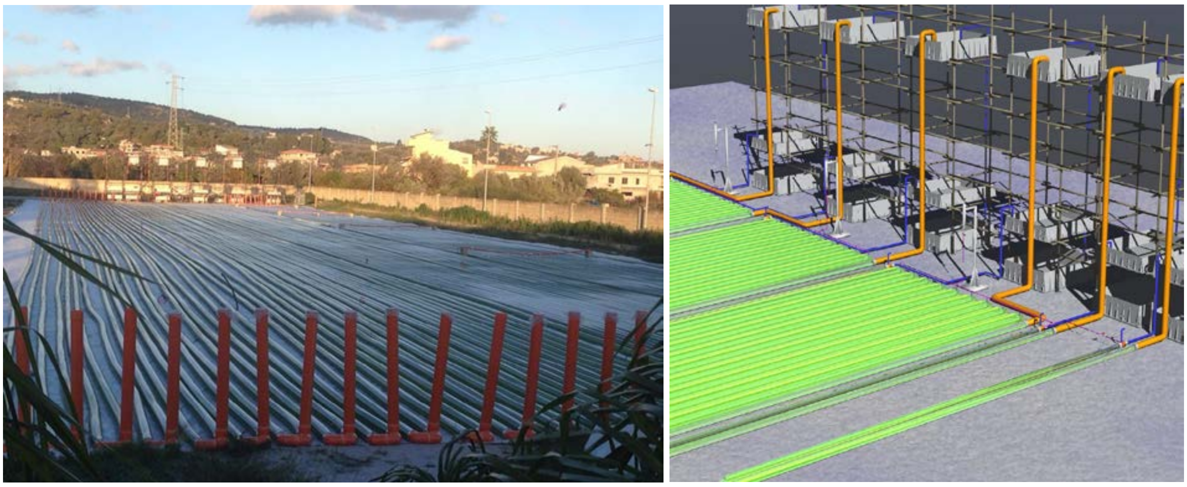

Figure 5: Algae cultivation plant