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COP21: The algae opportunity?

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ABSTRACT

Climate change is one of the main threats of the modern society. This phenomenon is associated with the increase of the greenhouse gases (GHGs, mainly carbon dioxide – CO_2) emissions due to anthropogenic activities. Main causes are the burning of fossil fuels and land use change (deforestation). Climate change impacts are associated with risks to basic needs (health, food security and clean water) as well as risks to development (jobs, economic growth and the cost of living). Taking into account this phenomenon, several countries participated in the last United Nations Climate Change Conference in Paris (21st Conference of the Parties – COP21) and agreed to reduce their GHG emissions to limit the rise in global temperature to less than 2 °C. Main commitments and actions are focused in energy efficiency, renewable energy deployment and forest protection (increasing the CO_2 natural sinks). In this context, biofuels (from non-edible feedstocks) have the potential to replace fossil based fuels in the transport sector, being a carbon-neutral fuel. In particular, algae–based biofuel can play a dual role in this scenario: as photosynthetic organisms, algae can capture CO_2 from industrial emissions or from atmosphere and the resulting biomass can be used to produce a wide range of materials including biofuels. Therefore, this paper reviews the research advances of algae cultures with focus on the applications (CO_2 capture and bioenergy production) related to the targets of COP21 agreement. Main recent advances in algal research studies and projects are also presented.

1. Introduction

Climate change is unequivocal [1–7]: (i) atmosphere and ocean have warmed (occurrence of heatwaves); (ii) the extents of snow and ice have decreased (Greenland and Antarctic ice sheets have been losing mass); (iii) sea level has risen (an average of 0.17 m since the beginning of the twentieth century), leading to coastal erosion, storm floods and flooding coastal areas; (iv) rainfall patterns has changed; and (v) greenhouse gas (GHG) concentrations have increased. Atmospheric carbon dioxide (CO_2, considered the most important GHG) concentrations have increased by 45% since the industrial revolution [8], mainly due to the increase of emissions from anthropogenic activities. Its value is now at more than 400 ppm corresponding to a carbon pool of around 850 Gt_C (considering a conversion factor of 2.12 Gt_C ppm^{-1}) [9–11]. The increase of atmospheric CO_2 concentration retains the heat emitted by Earth to space (changing the energetic balance on Earth), being correlated with the observed increase of the global average temperature (0.8 °C from 1880) [12]. Besides the global warming, the increase of atmospheric CO_2 concentration contributes to the ocean acidification, negatively affecting the biodiversity [13,14].

Therefore, climate change became one of the most relevant topics in the research activities and policy decisions. In 2015, several countries were represented in the 21st yearly session of the Conference of the Parties (COP21) that was held in Paris. The conference defined the Paris Agreement that comprises the policy actions in order to limit the impact of climate change. The main concern about the climate agreement is the economic impact [15]. There is a strong relationship between gross domestic product (GDP) and energy consumption that is now mainly obtained from fossil fuels. One of the most used models to describe this relationship is Kaya Identity [16]:

$$CDE = P\frac{GDP}{P}\frac{E}{GDP}\frac{CC}{E} - S_{CO_2} \quad (1)$$

where CDE is CO_2 emissions, P is the population, E is the energy production, CC is the quantity of carbon based fuels used for energy production, and S_{CO2} is the quantity of CO_2 removed by sinks. This model shows that the main drivers for the increase of CO_2 emissions are the population, GDP *per capita*, the energy intensity of the economy and carbon intensity of the energy system. The carbon intensity of the energy system is related on the type of usually consumed fuels. Its value depends on hydrogen to carbon (H/C) atomic ratio. High H/C ratio corresponds to low CC/E value (in other words, a "cleaner" energy) [15]: wood (H/C < 1); coal (H/C < 1); oil (H/C < 2); and natural gas (H/C < 4).

To fulfill the goals defined in Paris Agreement, the decarbonization

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of the global energy system should be studied, aiming to reduce the amount of CO_2 emitted to atmosphere. There are three main strategies [17,18]: (i) carbon reduction (enhancing energy efficiency and energy conservation); (ii) carbon capture and storage (CCS); and (iii) carbon abandonment (changing to zero-carbon energy sources and fuels, such as renewables and biofuels). Regarding the last option, biofuels gained much attention as they can replace fossil fuels without significant modifications of the current technology, contributing simultaneously to mitigate climate change. Its production from non-edible feedstocks avoids the competition between food and energy production markets. In this context, the algal culture can play a dual role [19–23]: as photosynthetic organisms, they are able to capture CO_2 and their biomass can be used to produce biofuels with zero or even negative net carbon balance. They present photosynthetic efficiencies ten times higher than terrestrial plants and an oil yield with one or two orders of magnitude higher than other biofuel feedstocks [24,25]. Consequently, algae culture represents a sustainable technology to limit the effects of climate change. This paper shows the recent research advances of algae production studies regarding CO_2 capture and bioenergy production. Special attention is focused on the basic findings achieved by main operational projects.

2. COP21

Global emissions of GHGs keep rising and, if no action is performed, the global average temperature should increase about 4 °C until 2100. Moreover, their residence time in atmosphere is long, which means that the global average temperature will rise even if GHG emissions are stopped (due to the inertia of the climate system). The current climate change is the consequence of cumulative GHG emissions since industrial revolution, when the unbalance between emissions and natural sinks was created.

Using paleoclimate data, modelling studies showed that an increase of 2 °C (regarding preindustrial level) is an acceptable mean temperature change target [17,26], corresponding to an atmospheric CO_2 concentration of 450 ppm (this value is now 407 ppm and it is increasing 2–3 ppm yr^{-1}). However, these models present high uncertainty, being recommendable to limit the increase to levels lower than 2 °C [27]. Thus, it is urgent the global agreement between countries about the reduction of GHG emissions. The Paris Agreement in December 2015 was an important step to fulfill this limit. Besides the reduction of GHG emissions, the conservation and enhancement of sinks of GHG (forests for instance) was incentivated. Essentially, the world should be carbon neutral: the emissions should be limited to a level that can be naturally absorbed by forest, oceans and soil. This carbon balance is expected to be achievable in the second half of this century. Comparing with other important climate agreement (Kyoto protocol) in which specific reduction targets were defined for developed countries, the Paris Agreement requires all countries to define their national determined contributions (NDCs), to delineate measures to fulfill their targets, and report the progress. These NDCs have indefinite duration, being revised every five years.

In terms of policy, it is recognized that European Union (EU) has the leadership role in combating climate change. In the last two decades, there were incentives to develop technologies to produce clean energy, such as solar photovoltaic, wind power, biomass and geothermal energy. Despite of significant cost reductions (due to the massive deployment of these technologies), the energy return on energy invested (EROI) is still below the value achieved with fossil fuels [28]. An EROI of 3 is considered the minimum value that a sustainable society must have. Regarding NDCs, EU and its Member States are committed: (i) to reduce at least 40% of GHG emissions by 2030 (when compared with 1990); (ii) to achieve at least 27% share for renewable energy; and (iii) to improve the energy efficiency by at least 27% [29]. This framework is intended to achieve a low-carbon economy with an energy system that guarantees: (i) cheap energy for all consumers; (ii) the improvement of EU energy security regarding suppliers; (ii) the reduction of dependence on energy imports; (iii) reduction of the balance of trade deficit; (iv) creation of new business opportunities and jobs; and (v) environmental and health benefits (for example, reduction of air pollution).

3. Biofuels as clean energy

Biofuels are considered one of the most promising alternative sources to fossil fuels, as its use does not imply significant changes in the current technological infrastructure. Thus, they are considered a short-term sustainable solution for the transport sector, reducing significantly the CO_2 emissions (at least 35%) when compared with fossil fuels [30,31]. Biofuels from biomass are quickly renewed. In addition, biomass can be produced from CO_2 (uptake from atmosphere) and water by photosynthesis, in which solar energy is converted into chemical energy. The carbon savings provided by biofuels depend on the used feedstock and the integrated processes selected for biofuel production. The first generation biofuels uses vegetable oil crops as feedstock. However, biofuel production yields from these sources do not achieve the current demand for fuels and the competition between energy and food markets for the feedstock led to the increase of food price [32,33]. Consequently, there was a negative public opinion regarding the first generation of biofuels due to the negative impacts on food security. The second generation biofuels are produced from crop and forest residues, and non-food energy crops (ligno-cellulosic feedstocks). Therefore, they are considered a more sustainable energy source, being however of limited scale [32,33]. The third generation biofuels are referred to the ones derived from algae. Initially, these biofuels were inserted in second generation category but they should be distinguished due to their high yields when compared with other feedstocks (algal area productivities are 10 times higher than the one achieved with best traditional feedstock) [20]. Algal biofuels include: (i) biodiesel; (ii) bioethanol; (iii) biomethane; (iv) bio-oil; and (v) bio-hydrogen. These biofuels have the potential to replace fossil-based fuels, being carbon neutral fuel. Algae are photosynthetic organisms that can be divided in three groups [34,35]: (i) microalgae; (ii) cyanobacteria; and (iii) macroalgae. Microalgae are eukariotic unicellular microrganisms. *Chlamydomonas reinhardtii*, *Chlorella*, *Dunaliella salina*, and diatoms are the most studied microalgae species for biofuel production. Cyanobacteria are prokaryotic unicellular microrganisms that presents high growth rates (they can double in 10 h). They do not usually present high oil content (for biodiesel production), but can be used to produce other type of biofuels. *Spirulina*, *Anabaena* and *Synechocystis* sp. are the most studied species. With high percentage of proteins (near 65%), they are commonly applied to food market. Macroalgae or seaweeds are multicellular eukariotic aquatic organisms that are abundant in oceans and coastal waters. Macroalgae are classified as *Phaeophyta* (brown algae), *Chlorophyta* (green algae), and *Rhodophyta* (red algae). Macroalgae do not only present high oil content (like cyanobacteria), but also high mass percentage of carbohydrates. In the next section, the technological issues are described for cultivation of microalgae, as they showed to be the most promising feedstock for biofuels production [15,36,37].

4. Algal cultivation

4.1. Bioreactors

Microalgae can be found in a wide range of habitats, including sewage, wastewater, desert, marine and sea water. Therefore, they can be cultivated within different environments and using different options, depending on the microalgal specie requirements and the value of the products that can be obtained from biomass [38]. Cultivation systems can be divided in two groups: open and closed ones.

Open ponds are the most commonly used system to cultivate

microalgae at large scale [39,40]. These systems present low capital and operational costs, being the suitable solution for biofuel production (to achieve a competitive price regarding fossil fuels). However, as an open system, the probability of contamination (with other species or bacteria or fungi) is high, causing variability on biomass quality and reducing the productivity. To reduce the impact of this phenomenon, cultivation of species resistant to severe environments is selected [20,41]. Besides contamination, local weather conditions strongly influences the parameters of the culture (temperature, for example). Water loss by evaporation and low CO_2 uptake efficiency (due to low mass transfer rate from gaseous to liquid phases) are other drawbacks of these systems [38]. Moreover, in autotrophic cultures, low photosynthetic efficiencies are achieved (near 3%) due to unefficient distribution of the light within the culture [42,43].

Closed systems (or photobioreactors – PBRs) are more interesting in terms of biotechnological engineering, as they allow to control almost all culture parameters, reducing contamination risk, water and CO_2 losses. Thus, it is easier to obtain reproducible cultivation and consequently the variability of biomass composition is reduced. The distribution of light within the culture is more efficient than open systems, achieving high photosynthetic efficiencies (around 6.5%) and biomass productivities [43]. However, capital and operational costs are high and these systems are only applied for high-value products (pharmaceutics, cosmetics, and food supplements). For biofuel production, these systems can only be used applying the biorefinery concept [44]. In other words, the maximum return from the biomass components should be achieved in these systems (converting them into marketable products), in order to reduce the price of produced biofuels and to contribute to a more economically and environmentally sustainable process [20,45]. The most common PBR designs are: (i) air-lift; (ii) bubble column; and (iii) flat plate (or panel). Air-lift PBR is characterized by high mass transfer rates, regular light/dark cycles, and low shear stress to the cultivated cells. The mixing of the culture is performed by bubbling the gas that forces the movement of the culture between the two sections of the bioreactor: riser and downcomer. Any other physical agitation is required in this reactor [41]. Bubble column PBR also presents high mass transfer rates, low energy consumption, and short circulation times. Mixing and CO_2 feed is provided by bubbling the gas from sparger. Flat plate PBR is characterized by high surface area to volume ratio, presenting the highest photosynthetic efficiencies and biomass yields. Mixing is also performed by bubbling air. Temperature control and hydrodynamic stress are the main disadvantages of these systems.

4.2. Key growth parameters

Microalgal growth is strongly influenced by several culture parameters: (i) nutrient quality and quantitative profiles; (ii) pH; (iii) temperature; (iv) light supply; (v) dissolved oxygen and CO_2; and (vi) presence of toxic elements in medium [46–49].

As with the terrestrial plants, microalgae requires some nutrients to grow. The main nutrients are carbon, nitrogen and phosphorus. Carbon is important for respiratory and photosynthetic metabolism. In autotrophic cultures, this nutrient is provided by CO_2 or dissolved carbonates, while heterotrophic microalgae use organic forms of carbon (glucose and acetate) [50]. Nitrogen is used for protein production and it has a strong influence on lipid and fatty acids profiles. Several researchers have already associated the effect of low concentration of this nutrient (stress condition) to the accumulation of intracellular lipids [49,51], which may be interesting for biofuel production. However, the lipid productivity decreases due to low microalgal growth rate in these environmental conditions [52]. Microalgae are able to assimilate inorganic forms of nitrogen, such as nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+); ammonium is the prefered source of nitrogen, as it can be directly assimilated, not requiring a previous reduction as nitrate (requiring more energy) [53]. Phosphorus is an important nutrient for photosynthesis, cell membrane and different microalgal metabolisms. It is assimilated from the medium in the phosphate form. Micronutrients are also required in microalgal growth media, such as some metals (e.g. Zn, Cu, Mn, Ni and Co). Other metals have unknown biological function and can be toxic for microalgae (e.g. Cd, Pb and Hg). Their concentration is essential to define their toxicity.

The pH of the culture can influence directly or indirectly the microalgal growth. This parameter influences the chemical equilibrium of the nutrients in the medium. Unsuitable values may reduce the availability of important nutrients for microalgae, reducing culture productivities [53]. Regarding direct impacts on microalgae, several metabolic processes are pH-dependent, such as enzyme activity processes (e.g. intracellular metabolism of nitrate) [54]. Variability in culture pH creates a gradient of pH between the cell and the medium. Thus, microalgae should adapt to this change by inducing a change in intracellular pH. Their ability to survive to these pH changes depends on the capacity to neutralize the new gradient of intracellular pH.

Temperature is other key parameter in microalgal growth. The optimal temperature for microalgae growth ranges between 15 and 26 °C. Temperature changes can induce stress in microalgae due to changes in cytoplasmatic viscosity, reducing the efficiency on carbon and nitrogen use. However, some microalgae have the ability to grow at higher temperature values (called thermo-tolerant), such as *Cyanidium caldarium*, *Galdieria partita* and *Cyanidioschyzon melorae* that exhibit an acceptable growth rates at 50 °C [55]. Moreover, low values are adverse for enzymatic activities, which are often associated with photosynthesis. Contrary, high temperatures inhibit the microalgal metabolic rate and reduce the CO_2 solubility [38].

For autotrophic cultures, light supply is one of the most important issues, as it constitutes the only energy source for microalgae (controlling the synthesis of energetic molecules). Light intensity should ensure an homogeneous distribution of light into the culture, in order to be available to all cells (including the ones located in lower layers). In high density cultures, high light intensity should be provided to minimize the effect of self-shading [56,57]. However, an excessive light intensity may cause photoinhibition. This phenomenon and the self-shading are the main causes for lower cell densities achieved by autotrophic cultures, when compared with heterotrophic cultures. Usually, the saturation light intensity increases with temperature [58]. Microalgal metabolism is enhanced, increasing light intensity up to 400 $\mu mol\ m^{-2}\ s^{-1}$.

5. Environmental risks of microalgal cultures

Microalgal cultures are associated to environmental risks. Four main concerns were already extensively discussed [59–61]: (i) water; (ii) land use; (iii) biodiversity; and (iv) GHG emissions. Microalgal cultures require high amount of freshwater and the addition of nutrients for culture contributes for the contamination of this resource. To avoid this problem, some research studies evaluated the effect of culture medium recycling on microalgal growth. Other option is the use of low quality water or wastewater as culture medium. In this case, the requirement for nutrients is significantly reduced, which is also an risk for microalgal culture. The massive culture can increase the demand for nutrients that can contribute to a new competition between food and energy markets (as in first generation biofuels). Moreover, autotrophic cultures require large culture areas, contributing to land use competition. Microalgal culture may influence local ecosystem, causing algal blooms and biological invasion. The evaluation of the environmental risks is especially hard for the cultures of non-native or genetically modified species [61], due to an unpredictable and fluctuating species balance. Another environmental risk is the GHG emissions (mainly CO_2, CH_4 and N_2O). At night and days with low irradiance levels, microalgae consume oxygen in respiration, causing anaerobic zones in PBR, leading to the emission of CH_4 and N_2O [59].

6. Algal as CO_2 sink

Algae play an important role in the balance of CO_2 in atmosphere. The photosynthesis that occur in the oceans is responsible for 40% of the overall amount of carbon annually fixed in the planet [62]. Thus, microalgae have a high potential to be an important CO_2 sink of anthropogenic emissions. Autotrophic microalgae are able to fix CO_2 from atmosphere and industrial flue gases. Their capacity to accumulate inorganic carbon in cytoplasm is one of the reasons to achieve high CO_2 uptake efficiencies [20,63,64]. Several research studies were performed to evaluate the CO_2 capture from a gaseous stream that is fed to a culture of microalgae. Table 1 presents CO_2 fixation rates or efficiencies for several species cultivated under different operational conditions.

The injection of enriched CO_2 gaseous stream in the culture promotes the dissolution of CO_2 in different forms (depending on the pH of the medium and in equilibrium) and enables the consumption of CO_2 by microalgae through photosynthesis. Sutherland et al. [65] studied the effects of CO_2 addition along a pH gradient on the nutrient removal from wastewater and the biomass production. In this study, the addition of CO_2 improved light absorption by microalgae. High biomass productivities were achieved when the pH of the culture was reduced from 8 to 6.5. However, nutrient removal efficiencies decreased, being associated with the decrease of chlorophyll-*a* in microalgal cells.

Each kilogram of microalgae corresponds to about 1.83 kg of fixed CO_2 [38,66]. Thus, the cost of CO_2 supply should be considered. Taking into account that CO_2 mass transfer rate is low, high amount of CO_2 should be fed to the culture. To reduce CO_2 loss to atmosphere, bioreactors should be designed to increase the CO_2 retention time, mainly in open ponds. Li et al. [67] constructed a closed raceway pond, covering a normal open raceway pond with a transparent cover, which directly touched the surface of the medium of microalgal culture. This coverage prevented the CO_2 escape to the atmosphere, increasing the CO_2 retention time. The results showed that the efficiency of CO_2 fixation increased to 95% under intermittent CO_2 supply.

Regarding CO_2 feed concentration, several researchers showed the ability of some microalgal species to grow at high concentrations [68,69]. High CO_2 levels led to the reduction of microalgal doubling times; however, there is an optimum value for each specie from which CO_2 becomes toxic for microalgal growth [38,70]. Boonma et al. [69] cultivating a consortium of microalgae (*Scenedesmus* spp., *Micractinium* sp., *Dictyosphaerium* sp., *Pseudanabaena* sp., *Monoraphidium* sp., *Chlamydomonas* sp., *Chlorella* sp. and *Euglena* sp.) with CO_2 at different concentrations (0.03%, 10% and 30%) and did not observe this toxic effect of CO_2 concentration. The results showed growth rates of 0.0163, 0.0068 and 0.0031 $g_{dw}\ L^{-1}\ d^{-1}$ for CO_2 concentrations of 30%, 10% and 0.03%, respectively. However, the chlorophyll content did not show the same tendency: the maximum chlorophyll content was observed for CO_2 feed concentration of 10%. Fulke et al. [71] evaluated the CO_2 fixation rates of four algal species, namely *Chlorella vulgaris*, *Scenedesmus obliquus*, *Chroococcus* sp. and *Chlamydomonas* sp., supplied with different CO_2 concentrations (1.4%, 3% and 7.5%). The results showed increases in the CO_2 fixation rates 3–10 times higher than the control, in which CO_2 was not supplied (atmospheric concentration). The CO_2 fixation rate of *Scenedesmus obliquus* and *Chlamydomonas* sp. increased from 0.1458 $g\ L^{-1}\ d^{-1}$ and 0.0347 $g\ L^{-1}\ d^{-1}$ to 1.813 $g\ L^{-1}\ d^{-1}$ and 0.3654 $g\ L^{-1}\ d^{-1}$, respectively, at 1.4% of CO_2; for *Chlorella vulgaris* increased from 0.0203 $g\ L^{-1}\ d^{-1}$ to 0.0708 $g\ L^{-1}\ d^{-1}$, at 3% of CO_2; the same happens for *Chroococcus* sp., which a 0.0121 $g\ L^{-1}\ d^{-1}$ increased to 0.127 $g\ L^{-1}\ d^{-1}$, at 7.5% of CO_2. It was concluded that photosynthetic CO_2 fixation was strongly reliant on the CO_2 concentration supplied during the period of algal growth.

7. Algal as biofuel feedstock

Microalgal biomass has several applications, such as human food, animal feed, cosmetic products, pharmaceutical products, fertilizers and biofuels [20,72,73]. Regarding biofuels, biodiesel and biomethane (biogas) production are the most studied processes, although the synthesis of bioethanol, biohydrogen and more recently bio-oil are also considered. Several studies have been proposed the simultaneous production of biodiesel and biomethane from microalgae [74–77]. In this process, anaerobic digestion of biomass is performed after lipid extraction.

Table 2 shows some recent studies regarding biofuel production from microalgae. From all the referred biofuels, biodiesel is the one that receives the most attention, as it presents similar physical and chemical characteristics of petroleum diesel [25]. Biodiesel has the advantage that it emits 78% less carbon dioxide when burned, 98% less sulphur, and 50% of particulate matter emissions [78]. Microalgae are well-known to synthesize and rapidly accumulate higher amounts of lipids in comparison to terrestrial plants, due to their high growth rates. Presently, the main challenges are related to the viability of large-scale commercialization of microalgae for biodiesel, since there are still some technical problems to overcome. Biodiesel production from microalgae includes several steps, such as cell cultivation and harvesting, oil extraction and biodiesel synthesis [79]. Although several attempts have been made to improve biodiesel yields from microalgae, further studies are required to improve biodiesel production rates and to reduce the energy consumption and the associated costs.

Microalgae are also a potential source of fermentable substrate. Some species presents high levels of carbon compounds, which are available (with or without a pre-treatment – enzymatic hydrolysis) for ethanol production. Besides fermentation, other bioethanol production process uses metabolic pathways in dark conditions, redirecting photosynthesis to produce hydrogen and alcohols. Silva and Bertucco [80] reviewed the main studies concerning bioethanol production from microalgae and cyanobacteria, focusing the impact of culture conditions on carbohydrates accumulation and the hydrolysis and fermentation yields obtained for different operating conditions. For application to industrial scale, the reduction of specific production costs is recommended, which can be achieved with increase of carbohydrate content in microalgae and biomass productivities.

Biomethane (one of the components of biogas – 65% methane and 35% of CO_2) is product of anaerobic digestion of organic matter. As referred above, microalgae biomass can be used as substrate. This biomass processing has an important advantage of non-requirement of the previous costly steps, such as microalgae harvesting and drying (that represents almost 30% to the production cost) [81]. In the last decades, two approaches of anaerobic digestion have been evaluated to produce biogas from microalgae: (i) through biomass and (ii) from lipid extracted biomass. Pre-treatment of biomass by chemical, enzymatic, mechanical or thermal processes can promote an increasing in methane yield [82].

Bio-oil is a microalgal biofuel that has recently attracted the attention of the research community, as it presents high energy density, low transport and storage risk when compared with gaseous fuels. Thus, bio-oil is considered a promising feedstock for replacement of petroleum fuel in power generation. However, it could not be used in transportation sector due to unfavourable physical and chemical properties, such as high viscosity, high oxygen content, high corrosiveness, and thermal instability. Bio-oil can be produced through biomass pyrolysis or thermochemical liquefaction [83].

Bio-hydrogen is considered the fuel of the future mainly due to its recyclability and non-polluting nature, because water is the unique combustion product. This biofuel leads high conversion efficiency (142 kJ/g). Microalgae used light energy to convert water to H_2 (biophotolysis) for photosynthesis [84]. The production of this biofuel requires anaerobic incubation of microalgae, to induce reversible

Table 1
CO_2 fixations rates of various microalgal strains under different operational conditions.

Reactor		Microalgae species	CO_2 supplied	Temperature	pH	Light conditions		Growth rate	CO_2 fixation		Ref.
Type	Volume (L)		(%)	(°C)		Intensity	Photoperiod	(d^{-1})	Rate	Efficiency (%)	
Open pond	5	*Chlorella kessleri*	0.038	30		39[c2]	12:12	0.17	1.09[c4]		[92]
	5	*Chlorella kessleri*	6	30		39[c2]	12:12	0.19	0.02[c4]		
	5	*Chlorella kessleri*	12	30		39[c2]	12:12	0.2	0.01[c4]		
	5	*Chlorella kessleri*	18	30		39[c2]	12:12	0.18	0.01[c4]		
	5	*Spirulina* sp.	0.038	30		39[c2]	12:12	0.12	0.67[c4]		
	5	*Spirulina* sp.	6	30		39[c2]	12:12	0.12	0.03[c4]		
	5	*Spirulina* sp.	12	30		39[c2]	12:12	0.13	0.01[c4]		
	5	*Spirulina* sp.	18	30		39[c2]	12:12	0.11	0.11[c4]		
	8	*Chlorella sp.*	10	30	10	30[c1]	12:12			46	[93]
	8	*Spirulina platensis*	10	30	10	30[c1]	12:12			39	
	12	*Scenedesmus obliquus*	15	24 ± 1		100[c1]				56	[67]
	330	*Chlorella sp.*	6–8	31–34	7–8	Sunlight				50	[94]
	20,000	*Scenedesmus sp.*	10.6		8	Sunlight				66	[95]
	20,000	*Scenedesmus sp.*	2–6		8	Sunlight				85	[96]
	20,000	*Scenedesmus sp.*	14		8	Sunlight				32	
Air-lift PBR	1.4	*Anabaena* sp.	air	30		120[c2]		0.87	0.346[c4]		[97]
	1.4	*Anabaena* sp.	5	30		120[c2]		1.15	0.587[c4]		
	2.4	*Aphanothece m. Nägeli*	15	25		150[c1]	12:12		14.5[c4]		[98]
	4	*Chlorella sp.*	10	26 ± 1		300[c1]		0.252		63	[99]
	100	*Scenedesmus obliquus*	10	26		12,000[c3]				40.2	[100]
	1000	*Tetraselmis suecica*	11	25	7.5	Sunlight			0.0892		[101]
Bubble column reactors	0.5	*Chlorella vulgaris*	10–13	30	6.5–7.5	1150[c2]			4.4[c4]		[102]
	0.6	*Chlorella sp.*	5		7.2	100[c1]			0.58[c4]		[103]
	0.8	*Chlorella sp.*	2	26 ± 1		300[c1]			7.83[c4]	58	[104]
	0.8	*Chlorella sp.*	5	26 ± 1		300[c1]			9.48[c4]	27	
	0.8	*Chlorella sp.*	10	26 ± 1		300[c1]			14.0[c4]	20	
	0.8	*Chlorella sp.*	15	26 ± 1		300[c1]			17.2[c4]	16	
	1	*Scenedesmus obliquus*	2.5	24		210–230[c1]		1.501	0.803[c4]		[105]
	1	*Scenedesmus obliquus*	2.5	29		210–230[c1]		1.609	1.002[c4]		
	1	*Scenedesmus obliquus*	2.5	34		210–230[c1]		1.702	1.030[c4]		
	1	*Scenedesmus obliquus*	2.5	30		210–230[c1]		1.412	1.608[c4]		
	1	*Thermosynechococcus sp.*	10	55		10,000[c3]	24:00	2.7			[106]
	1.4	*Anabaena* sp.	air	30		120[c2]		0.45	0.28[c4]		[97]
	1.6	*Chlorella vulgaris*	0.2		9 ± 0.5	40–50[c1]			1.53[c4]	74	[107]
	1.8	*Anabaena sp.*	air	27	8.5	900[c2]			1.45[c4]		[108]
	2	*Aphanothece m. Nägeli*	15	30		150[c1]	24:00:		26.9[c4]		[109]
	2.4	*Aphanothece m. Nägeli*	15	25		150[c1]	12:12		13.0[c4]		[98]
	2.4	*Chlorella vulgaris*	13	25		180		1.1 ± 0.2	0.98[c4]		[54]
	3	*Aphanothece m. Nägeli*	15	35		11,000[c3]	24:00		2.621[c4]		[110]
	3	*Aphanothece m. Nägeli*	15	35		150[c1]			1.44[c4]		[111]
	5.4[a]	*Spirulina sp.*	6	30		3200[c3]	12:12			37.9	[112]
	8	*Botryococcus braunii*	5	25	7.2	3500[c3]	12:12		0.497[c4]		[113]
	8	*Dunaliella tertiolecta*	5	25	7.2 ± 0.2	3500[c3]	12:12		0.272[c4]		
	8	*Spirulina platensis*	5	30	9.0 ± 0.2	3500[c3]	12:12		0.319[c4]		
	8	*Chlorella vulgaris*	5	30	7.2 ± 0.2	3500[c3]	12:12		0.252[c4]		
	10[b]	*Chlorella vulgaris*	1	25–30		157.6[c2]	12:12		6.24[c4]		[114]
Flat-plate PBR	11.4	*Chlorococcum littorale*	5	25	6.1–7.2	2000[c1]			200.4[c5]		[115]
	72	*Synechocystis aquatilis*	10	40 ± 3		sunlight			51[c5]		[116]

(continued on next page)

Table 1 (*continued*)

Reactor		Microalgae species	CO_2 supplied	Temperature	pH	Light conditions		Growth rate	CO_2 fixation		Ref.
Type	Volume (L)		(%)	(°C)		Intensity	Photoperiod	(d^{-1})	Rate	Efficiency (%)	
Other PBR	1.8	*Chlorella kessleri*	6	30		3200[c3]	12:12	0.267			[117]
	1.8	*Scenedesmus obliquus*	12	30		3200[c3]	12:12	0.261			
	100	*Euglena gracilis*	5–10	27	3.5 ± 0.1	178.7[c1]			0.074[c4]		[118]

Blank indicates no information available.
PBR: Photobioreactor
[a] Three-stage serial bubble column reactor (3*1.8 L).
[b] Bubble column reactor with membrane.
[c1] in $\mu mol\ m^{-2}\ s^{-1}$.
[c2] in $\mu E\ m^{-2}\ s^{-1}$.
[c3] in lux.
[c4] in $g\ L^{-1}\ d^{-1}$.
[c5] in $g\ m^{-2}\ d^{-1}$.

hydrogenase which combines protons and electrons to form hydrogen; however, microalgal hydrogenase is sensitive to oxygen and so it is easily deactivated by the oxygen which is evolved during photosynthesis. The sulphur deprivation of cells is one of the strategies to overcome this inhibition, because it leads a reversible deactivation of photosystem II, which results that oxygen evolution is stopped [85]. The bio-hydrogen production from microalgae have been reported in *Anabaena* sp., *Nannochloropsis* sp. and *Scenedesmus obliquus* [84].

8. Projects

In this section, the description of the main recent research projects developed in European Union (EU) and United States are described. Table 3 presents the main information from European research projects regarding biofuel production by microalgae.

8.1. European Union

All the research projects described below were funded by the 7th Framework Program (FP7) for research and technological development. It was the EU primary instrument for funding research and demonstration activities from 2007 through 2013. The total budget for the seven-year period was €51 billion, in which €2.35 billion was kept for energy-related projects.

AquaFUELS (Algae and aquatic biomass for a sustainable production of 2nd generation biofuels) aimed to draw the detailed, comprehensive and concrete picture of the actual status quo of EU and international initiatives on algal biofuels. Major research and industrial needs were identified and full life cycle analysis was performed. The project provided the support for the establishment of the European Algae Biomass Association, having as main objective the promotion of cooperation in the field of algal biomass research, production and use.

In 2011, three projects (Algae Cluster) were funded, focusing large scale demonstration of biofuels production from algae with ambitious and achievable targets: (i) minimum plantation area of 10 ha; and (ii) minimum productivity of 90 dry solid tons per hectare per year. **BIOFAT** (BIOfuel From Algae Technologies) integrated the entire value chain of algal production from the growth optimization to downstream processing (biorefinery approach). This demonstration project aimed to assess large scale microalgal production focusing energy efficiency, economic viability and environmental sustainability. BIOFAT consortium combined knowledge and expertise in the field of microalgal production and applications. **All-Gas** (Industrial scale demonstration of sustainable algae cultures for biofuel production) project aimed to demonstrate the sustainable production of low-cost biofuel from algae, based on the reuse of wastewater and other residues. Therefore, it was based on nutrient recycling, energy harvesting and CO_2 generation from wastewater and its residues. After anaerobic treatment, wastewater was purified for the growth of algae. Algae were then harvested and processed for oil extraction. The residual biomass was transformed (by anaerobic digestion) into methane, CO_2 and minerals, together with other biomass from wastewater and/or agriculture residues. This project introduced a newly patented device, the Light Enhancement Factor, to significantly increase the biomass yield of raceway ponds (maintaining the positive energy balance). **InteSusAl** (Integrated Sustainable Algae) aimed to generate biofuels from algae in a sustainable manner (in terms of both economic and environmental – closed carbon loop – implications) on an industrial scale. This project focused on the optimization of algal production by both heterotrophic and phototrophic routes, having cultivation targets of 90–120 dry tonnes per hectare per year. Algal species and cultivation technologies **were** selected according to their lipid profile for biodiesel production, in which selection was subsequently validated through conversion of extracted oil into biodiesel to meet standard specifications.

EnAlgae (Energetic Algae) was a four-year strategic initiative of the

Table 2
Biofuel conversion process using microalgae as feedstock.

Biofuel	Microalgae	Process description	Results	Ref.
Biodiesel	*C. vulgaris*	**R**: Acid-catalysed esterification + Base-catalysed transesterification; Microwave reactor; CC = 0.5–2.5% (wt/v); L/M ratio = 0.07–0.25; T = 45–65 °C; t = 5–25 min **C**: ^{1}H NMR; ^{13}C NMR; FTIR; GC.	**Y**: 84.01% **EP**: 13.62 t ha^{-1} yr^{-1}	[119]
	Scenedesmus obliquus	**R**: Transesterification; T = 35 °C, L/M ratio = 0.2. **C**: Fuel properties (ASTM 6751; EN14214).	**Y**: 90.8 ± 1.4%	[120]
	Nannochloropsis sp.	**R**: Direct transesterification; ionic liquid (solvent); T = 65 °C; t = 15 min **C**: GC.	**Y**: 36.79%	[121]
Bioethanol	*S. dimorphus* (lipid extracted biomass)	**R**: Saccharification and fermentation; amyloglucosidase enzyme – 60 units/ml; *Sacchromyces cerevisiae* – 3 g L^{-1}; pH = 5, T = 36 °C.	**Y**: 0.26 g ethanol g CDW^{-1}	[122]
	Chlorella vulgaris	**R**: Fermentation; bacterium *Z. mobilis* ATCC 29191; T = 30 °C.	**Y**: 87.59%	[123]
	Synechococcus sp.	**R**: Fermentation; *Saccharomyces cerevisiae*; V = 10 ml; T = 34 °C. **C**: HPLC.	**Y**: 0.27 g ethanol g CDW^{-1}	[124]
	Chlorella sp. (lipid extracted biomass)	**R**: Fermentation; *S. cerevisiae* KCTC 7931; V = 0.5 l; T = 30 °C; pH = 6. **C**: HPLC.	**Y**: 0.16 g ethanol g CDW^{-1}	[125]
Biomethane	*C. vulgaris, S. obliquus, C. reinhardtii*	**R**: Anaerobic digestion; Batch reactor; V = 0.070 L.**C**: GC.	**Y**: 106–171 ml CH_4 g COD^{-1} **BD**: 30–49%	[126]
	Tetraselmis spp.	**R**: Anaerobic digestion; Batch reactor; V = 0.5 L **C**: GC.	**Y**: 252 ml g VS^{-1} **BD**: 65–66%	[127]
	C. vulgaris	**R**: Fermentation; enzymatic pre-treatment; Batch reactor; V = 0.25 l; T = 37 °C. **C**: GC; HPLC.	**Y**: 245–414 ml CH_4 g VS^{-1}	[128]
Bio-oil	*Cyanobacteria* sp.	**R**: Hydrothermal liquefaction; Batch reactor; T = 250–350 °C; t = 15–90 min **S**: Filtration + Solvent extraction (CH_2Cl_2). **C**: Elemental composition analysis; GC-MS; FTIR; NMR; TGA; TOC; TN.	**Y**: 21.10% (wt). **H/C ratio**: 1.37–1.62.	[129]
	Nannochloropsis oceanica	**R**: Hydrothermal liquefaction; Batch reactor; T = 240–300 °C; P = 32–89 bar. **C**: Elemental composition analysis; GC-MS.	**Y**: 54 ± 2%	[130]
	Nannochloropsis sp.	**R**: Hydrothermal liquefaction; Batch reactor; T = 350 °C; t = 2 min	**Y**: < 37% (wt).	[131]
Biohydrogen	*C. vulgaris*	**R**: Dark fermentation; enzymatic pre-treatment; Batch reactor; V = 0.25 l; T = 60 °C. **C**: GC; HPLC.	**Y**: 19–135 ml H_2 g VS^{-1}	[128]

R – reaction; S – separation; C – characterization; GC-MS – gas chromatography–mass spectrometry; FTIR – Fourier transform infra-red; NMR – nuclear magnetic resonance; TGA – thermogravimetric analysis; TOC - Total Organic Carbon; TN – total nitrogen; Y – yield; H/C – hydrogen to carbon; BD – Biodegradability; EP – estimated productivity; CC - catalyst concentration; L/M – lipid to methanol; CDW – cell dry weight.

INTERREG IVB North West Europe (NWE) program. This project aimed to assess the potential of energy and fuel productions from both microalgae and macroalgae, reducing CO_2 emissions and dependency on unsustainable energy sources in NWE. Specific objectives included: (i) the development of a network of pilot and demonstration sites, identifying factors for optimization of algal cultivation environment; (ii) the assessment of technical and economic feasibility of algal production in the studied region (NWE); and (iii) the SWOT analysis to identify the opportunities and barriers for producing energy from algae.

ALGADISK (Novel algae-based solution for CO_2 capture and biomass production) aimed to develop a modular, scalable, and automatic biofilm reactor for algal biomass production, with low operational and installation costs. The reactor was designed to capture CO_2 from industrial emissions to produce high value organic products. Automatic and continuous harvesting of algae was designed to optimize CO_2 uptake and biomass production. The proposed system was specifically crafted to meet the needs of European SMEs who are willing to produce algal biomass products from industrial emissions.

DEMA (Direct Ethanol from MicroAlgae) aims the development of a

Table 3
Description of main European research projects regarding biofuel production from algae.

Project	Budget / Period	Consortium	Focus areas	Ref.
AquaFUELS	€0.87 M 2010–2011	13 partners (BE; CZ; ES; FR; IL; IR; IT; NL; PT; UK)	Biofuel sustainability evaluation regarding CO_2 balance, land and water use, and competition with food.	[132,133]
BIOFAT	€10 M 2011–2015	7 partners (ES; FR; IL; IT; NL; PT; US)	Integration of the entire value chain of algae process from optimized growth, starch and oil accumulation, to downstream processing (biorefinery).	[133–135]
All-Gas	€11.8 M 2011–2016	7 partners (AT; DE; ES; NL; TR; UK)	Demonstration of the sustainable large-scale production of biofuels based on the low-cost cultivation of microalgae (wastewater use).	[134]
InteSusAl	€8.6 M 2011–2015	11 partners (BE; DE; NL; PT; TR; UK)	Demonstration of an integrated approach to generate biofuels from algae in a sustainable manner on an industrial scale (autotrophic and heterotrophic routes).	[134,136]
EnAlgae	€14.6 M 2011–2015	19 partners (BE; DE; FR; IR; NL; UK)	Development of algal bioenergy technologies at nine pilot facilities and search for opportunities in the emerging marketplace in North West Europe.	[137–149]
ALGADISK	€3.2 M 2012–2014	12 partners (BE; ES; HU; NL; SI; TR; UK)	Development of a modular, scalable, and automatic biofilm reactor for algae biomass production, with low operational and installation costs.	[150–152]
DEMA	€6.4 M 2012–2017	10 partners (FI; FR; IR; NL; PT; UK)	Demonstration and licensing of a complete economically competitive technology for the direct production of bioethanol from microalgae with low-cost scalable PBRs.	[153]
FUEL4ME	€4 M 2013–2016	11 partners (AT; DK; ES; IL; IR; IT; NL)	Development and demonstration of an integrated and sustainable process for continuous biofuel production from microalgae, aiming to fulfill European climate and energy goals.	[154,155]
MIRACLES	€11.9 M 2013–2017	26 partners (BE; DE; ES; GR; NL; PT; Others)	Development of an environmentally friendly, integrated biorefinery technologies for production of specialties from algae for application in food, aquaculture and selected non-food applications.	

AT – Austria; BE – Belgium; CZ – Czech Republic; DE – Germany; ES – Spain; FI – Finland; FR – France; GR – Greece; HU – Hungary; IL – Israel; IR – Ireland; IT – Italy; NL – Netherlands; PT – Portugal; SI – Slovenia; TR – Turkey; UK – United Kingdom; US – United States of America.

competitive technology for direct production of bioethanol from microalgae with low-cost scalable photobioreactors. Initial proof-of-concept results show that it is feasible to use microalgae to produce bioethanol for less than €0.40 per litre. The DEMA bioethanol process is economically, socially, and environmentally positive, providing a complement and future replacement to terrestrial biomass-derived ethanol and act as an immediately actionable means of reducing the carbon footprint of EU transport needs. The DEMA consortium aims to achieve a Transformational Innovation in biofuel production via low risk improvement of existing technologies at proof of concept stage.

FUEL4ME (FUture European League 4 Microalgal Energy) aimed to design a sustainable chain for continuous biofuel production using microalgae, reducing CO_2 emissions and finding an alternative to fossil fuels. The study was initially performed in indoor conditions and then outdoor tests were expected to evaluate real production in Spain, Italy, Netherlands, and Israel. Finally the whole process were integrated and subjected to an economic and life cycle analysis.

MIRACLES (Multi-product Integrated bioRefinery of Algae: from Carbon dioxide and Light Energy to high-value Specialties) is an industry-driven R & D and innovation project funded by FP7. It aims at developing integrated, multiple-product biorefinery technologies for the production of specialties from microalgae for application in food, aquaculture and non-food products. The focus is on the development and integration of mild cell disruption and environmentally-friendly extraction and fractionation processes, including functionality testing and product formulation based on established industrial algal strains. The project also aims to develop new technologies for optimizing and monitoring valuable products in the algal biomass during cultivation.

8.2. United States

Twelve projects aiming the assessment of innovative concepts for the beneficial use of carbon dioxide were funded by the United States Department of Energy (American Recovery and Reinvestment Act – ARRA). These concepts included: (i) the mineralization of CO_2 from flue gases to carbonates; (ii) the use of CO_2 to grow algae/biomass; and (iii) conversion of CO_2 to fuels and chemicals. Each project was then evaluated to determine the ones that would be funded for design, construction and testing of pilot systems (second phase). The funding in initial phase was $25.1 M ($17.4 M from ARRA and $7.7 M from private funding). Five of the twelve selected projects were related to algal cultivation. Gas Technology Institute and partners proposed the capture of CO_2 with macroalgae (seaweeds) cultivated in nonsubmerged greenhouses. Macroalgae were then harvested and used to produce methane (by anaerobic digestion) for fuel to a power plant. Phycal LLC and partners designed a process for CO_2 capture and use for cultivation of microalgae in open ponds (wastewater was used as culture medium). Microalgal biomass was then processed to produce oil. Sunrise Ridge Algae and partners tested the cultivation of algae using CO_2 from cement plant. Algae was then harvested and converted into liquid fuel and carbonaceous char using catalytic thermochemical conversion technology. Touchstone Research Laboratory and partners cultivate algae in raceway ponds using CO_2 from combustor flue gas. Lipids were extracted from algal biomass to produce biofuel and this biomass was used to produce electricity and recover nutrients. UOP LLC and partners tested a Vaperma membrane to capture CO_2 from a caprolactam production plant. Captured CO_2 was fed to microalgal cultures, whose biomass was then processed to biofuel and fertilizer. In 2010, the six most promising projects were selected for the second phase with total funding of $82.6 M. Two of the projects above referred were selected in this phase. Phycal, LLC and partners developed an integrated system to produce liquid biocrude fuel from microalgae cultivated with captured CO_2. Algal fuel can be blended with bother fuels for power generation. The proposed tasks were the design, build and operate a microalgal cultivation facility at a nominal thirty acre site in Hawaii. Touchstone Research Laboratory and partners designed a pilot scale open pond to capture at least 60% of flue gas from an industrial coal-fired plant and the produced algae was then used to produce biofuel and other high-value products. The open ponds designed by Touchstone can regulate the daily temperature, reduce water evaporation and control the contamination of the cultures. Lipids extracted from algae were processed to produce biofuel. Anaerobic digestion process was developed and tested to convert residual biomass into methane. The pilot unit was tested in Ohio.

The Department of Energy Office of Energy Efficiency and Renewable Energy's (DOE-EERE's) Biomass Program awarded $48.6 M (funding from ARRA; 2010–2013) for the NAABB (National Alliance for Advanced Biofuels and Bioproducts) consortium. NAABB combined expertise from the national laboratories, universities and industries. This consortium consisted of 39 institutions and had two international partners. Its main objective was to develop research to break down critical technical barriers to commercialization of algae-based biofuels. All different steps of algal biofuels production were focused: microalgal strain selection, harvesting, oil extraction, fuel conversion and agriculture co-product production [86–91]. The technology developments were evaluated according to their sustainability and financial feasibility. Thus, NAABB technologies focused on the reduction of nutrients and water requirements for algal cultivation and the simultaneous reduction of carbon footprint. If key innovations of NAABB were implemented along the entire value chain, the cost of algal biofuels could be reduced from starting baseline of $240 per gallon to a reasonable cost of less than $7.50 per gallon of crude oil.

9. Future trends

CO_2 capture with microalgal cultures may be a sustainable solution to reduce the emissions of this gas to atmosphere. In addition, the resulting biomass can be used for several applications, including biofuels which can contribute to the reduction of fossil fuels consumption (and associated CO_2 emissions). However, research efforts should be performed in different sectors of microalgal production. High requirement of nutrients for microalgal growth may compromise the sustainability of the process. However, wastewaters can be used as microalgal culture medium, reducing the requirement of chemical fertilizers. Research studies should be performed to evaluate the integration of these processes in order to optimize the microalgal growth and to achieve pollutant concentrations in the final effluent with values lower than the standard ones for the protection of the environment. Another issue to be studied is the solubility of CO_2 in the medium. Low values lead to the loss of this gas to atmosphere before being able to be up-taken by microalgae. The use of membranes in microalgal cultivation may optimize the use of CO_2. Moreover, the economic value of the process can be improved with the integration of the environmental applications (CO_2 capture and wastewater treatment) and the optimization of the microalgal biomass use (in a biorefinery concept).

10. Conclusions

This paper presented a review of the research advances of algae production regarding CO_2 capture and bioenergy production, applications related to the targets of COP21 agreement. The current research activities are focused on: (i) process integration (CO_2 capture, wastewater treatment and biofuel production) in order to reduce the overall cost; (ii) optimization of bioreactors design to improve the CO_2 capture efficiency and biomass areal productivities; (iii) test of different processes to improve biofuel yield, assessing the impact of process variables on biofuel properties; and (iv) extraction of valuable products from algal biomass (biorefinery concept) to reduce the cost of biofuel. This paper also highlights the environmental risks associated with microalgal production and options to mitigate them. Regarding EU and US research projects, the scale-up of the technology is evaluated,

analysing the energy efficiency, economic viability and environmental sustainability. Achieved technological advances led to determine a biofuel market value that can compete with fossil based fuels with less environmental impact.

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