



## Optimization of light use efficiency for biofuel production in algae



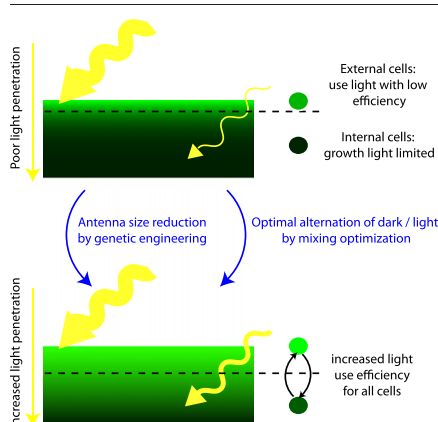
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### HIGHLIGHTS

- Algae have interesting potential for the production of biofuels.
- Light use efficiency is one of the major factors influencing algae productivity.
- Investigation of molecular bases influencing photochemical efficiency is seminal to optimize algae productivity.
- Productivity can be improved by genetic engineering and optimization of photobioreactor operational parameters.

### GRAPHICAL ABSTRACT



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### ABSTRACT

A major challenge for next decades is development of competitive renewable energy sources, highly needed to compensate fossil fuels reserves and reduce greenhouse gas emissions. Among different possibilities, which are currently under investigation, there is the exploitation of unicellular algae for production of biofuels and biodiesel in particular. Some algae species have the ability of accumulating large amount of lipids within their cells which can be exploited as feedstock for the production of biodiesel. Strong research efforts are however still needed to fulfill this potential and optimize cultivation systems and biomass harvesting. Light provides the energy supporting algae growth and available radiation must be exploited with the highest possible efficiency to optimize productivity and make microalgae large scale cultivation energetically and economically sustainable. Investigation of the molecular bases influencing light use efficiency is thus seminal for the success of this biotechnology. In this work factors influencing light use efficiency in algal biomass production are reviewed, focusing on how algae genetic engineering and control of light environment within photobioreactors can improve the productivity of large scale cultivation systems.

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### 1. Introduction

The largest fraction of world energy demand is presently met by the combustion of coal, oil and natural gas. Such a massive

exploitation of fossil fuels leads to the release of large amounts of carbon dioxide and other pollutants in the atmosphere with detrimental effects on the environment. Also, because of this massive consumption, global reserves will be depleted in the future. It is thus evident that there is a strong need of alternative, renewable and environmentally compatible sources of energy in order to sustain our present lifestyle [1]. Among different possibilities, photosynthetic organisms are receiving growing

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attention for their potential exploitation in the production of biofuels [2–6]. These bio-derived compounds in fact represent one of the most promising sources of liquid fuels, which are extensively used for transportation and in some cases, such as for jets, are not replaceable by electricity with the present technology.

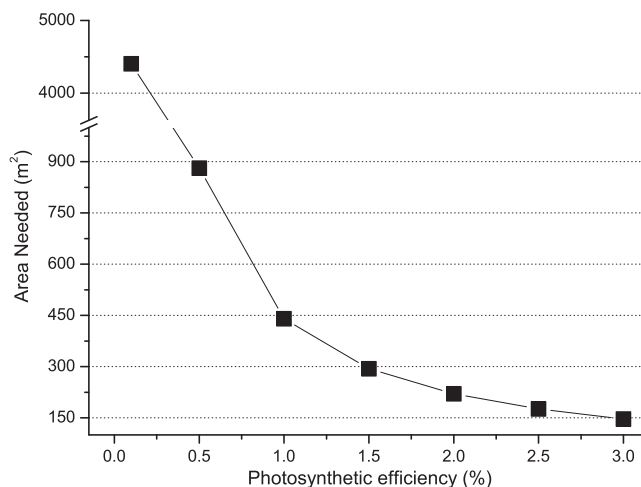
In this direction, a major potential alternative to fossil fuels for transportation is biodiesel which can be produced from vegetal oil through a process of trans-esterification. Biodiesel production on a large scale, however, is at present strongly limited by the feedstock supply. Nowadays, most biodiesel is produced from oils extracted from crops like soy and palm, which have a limited productivity and would demand unrealistic areas of cultivation in order to replace a substantial fraction of fossil fuels [7,8]. A further critical point is that crop plants are normally employed as food or feed and their exploitation for biodiesel will generate an undesirable competition for cultivation areas and fresh water [9].

One interesting alternative to crops is the exploitation of other photosynthetic organisms such as microalgae, which are capable of accumulating large amounts of lipids which can be extracted, processed and refined into transportation fuels [4,6]. Algae also have additional interesting features such as the ability to efficiently use CO<sub>2</sub> [4] and, at least for some species, fast growth rate [10–14]. The production of biofuels can also be combined with the use of algal systems for wastewater treatment to reduce the carbon, nitrogen and phosphorus content in industrial, municipal and agriculture wastes [15,16]. Furthermore, microalgae-derived high added value molecules can be used in the cosmetic or food industry such as astaxanthin, β-carotene, omega-3-fatty acids, vitamin E and other pigments [16–19].

While it is thus clear in the scientific community that algae are highly promising for biofuel production and other applications, intensive research efforts are still needed to exploit their potential in large scale cultivation systems [5,6,20]. Many factors influence algae growth and productivity and deeper investigations are necessary to optimize operating parameters in large scale algae cultivation systems (photobioreactors, PBRs) and maximize their productivity (for a comprehensive review see [4,21]). One of the major factors affecting algae growth is light: as for all photosynthetic organisms, sunlight provides the energy supporting their metabolism and its efficient conversion into biomass has a major influence on productivity. The importance of this parameter is exemplified in Fig. 1, where the area needed to produce a ton of biomass per year is represented depending on the energy conversion efficiency. For values as low as 0.1%, the average value for most crop plants in field conditions [22], the requested area is very large, while this is drastically reduced if photosynthetic efficiency reaches 3%, a value experimentally obtained with algae in laboratory conditions [23]. Possible improvements could eventually further increase the biomass productivity, closing the gap with the maximal theoretical efficiency ( $77 \pm 5 \text{ g dw m}^{-2} \text{ d}^{-1}$ , corresponding to  $\approx 12\%$  efficiency [24]).

It should be underlined that, while crop plants are routinely cultivated in large areas, algae are cultivated in photobioreactors or ponds which have high energetic and monetary cost for building and maintenance. Therefore any increase in the area occupied by the alga cultivation makes the process less sustainable from the energetic and economic point of view. Therefore, a high photosynthetic efficiency is indispensable for a viable algae large scale cultivation system, even more than for crop plants. For this reason a deeper understanding of the molecular bases of the light use efficiency for these organisms is seminal to optimize their cultivation on a large scale and will be the focus of the present work.

Although it is unlikely that a single species will have all the optimal characteristics for biodiesel production in all conditions, the species belonging to the genus *Nannochloropsis* are receiving increasing attention for this kind of applications. In fact, they present several positive features such as good growth rates and the ability to accumulate large amounts of lipids, up to 60% of total dry weight [14,25,26]. Recent



**Fig. 1.** Estimation of area needed for alga production. The area occupied by an alga cultivation system producing 1 ton of dry biomass per year is shown in dependence of the light use efficiency. Average radiation intensity was assumed to be  $4541 \text{ MJ m}^{-2} \text{ y}^{-1}$  (data for Padova, Italy, according to Photovoltaic Geographical Information System, PVGIS Solar Irradiation Data, 2007, <http://sunbird.jrc.it/pvgis/>) and biomass energy content was assumed to be  $20 \text{ kJ/g}$ .

availability of genome sequences and tools for their molecular modification is also contributing to make this species a model for the study of biofuel production from algae [27,28], complementing the studies on other model organisms such as the green alga *Chlamydomonas reinhardtii* which is better characterized but less efficient as lipids producer. For this reason, *Nannochloropsis* and *Chlamydomonas* will be used here as the main reference species, although major conclusions are most likely valid for other species as well.

## 2. Influence of light intensity on photosynthetic efficiency

Algae grown in large scale cultivation systems, such as PBRs, are exposed to a complex light environment. First of all sunlight is not constant but its intensity continuously changes during the day and the seasons. Illumination intensity has an important influence on alga productivity, as shown in Fig. 2 for the case of *Nannochloropsis salina*: up to  $150 \mu\text{mol of photons m}^{-2} \text{ s}^{-1}$  an increase in illumination stimulates growth, showing that, in this range of intensities, available light is the limiting factor. Once this limit is surpassed, however, growth is not stimulated anymore by an increase in light intensity but, on the contrary, it has an inhibitory effect, causing reduction in duplication rate [29]. It is important to underline that, in the experiments reported here, *Nannochloropsis* cells were cultivated in a flat-bed PBR in order to expose all cells to the same irradiation, reducing as much as possible the cells' self-shading. Also, carbon dioxide and nutrients were provided in excess to highlight the influence of light regime on growth kinetics.

Similar experiments, performed at atmospheric CO<sub>2</sub> concentration, showed different results and light was limiting in a much smaller interval, only below  $15 \mu\text{mol of photons m}^{-2} \text{ s}^{-1}$  [30]. In these conditions, irradiation between  $15$  and  $150 \mu\text{mol of photons m}^{-2} \text{ s}^{-1}$  has little influence on duplication rates suggesting that, in this case, growth is limited by CO<sub>2</sub> supply (Fig. 2). The importance of this key substrate for algae growth is well established and in fact all large scale PBRs are normally designed to provide cells with additional CO<sub>2</sub> supply. Actually, the ability of algae to exploit high carbon dioxide supplies represents a major advantage of these organisms and opens the possibility of cultivating algae in connection with industrial processes which produce large amounts of CO<sub>2</sub>. Such a combination, in fact, while providing a low cost CO<sub>2</sub> source for algae cultivation allows fixing part of it into biomass, thereby reducing emissions in the atmosphere.

For the above mentioned reasons, the influence of light regime on algae performances in a large scale PBR should be studied under

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