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Photobioreactors for microalgal growth and oil production with *Nannochloropsis salina*: From lab-scale experiments to large-scale design

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A B S T R A C T

This paper reports new experimental data of microalgae growth and lipid production under autotrophic conditions for the species *Nannochloropsis salina*. The effect of relevant operating variables is addressed and discussed, and some suggestions to better understand the process behavior are given with respect to lipid content maximization, carbon dioxide and nitrogen supply, and illumination conditions. The data obtained are finalized to the design of an environmentally and economically sustainable photobioreactor in view of achieving industrial photosynthetic biomass and natural oil production from large scale microalgae cultivation.

Keywords: Microalgae; Biomass; Bio-oil; Industrial photosynthesis; Photobioreactor; Second-generation biodiesel

1. Introduction

The interest in microalgae cultivation is currently very high because microalgal oil, among other uses, could represent an alternative to complement and eventually replace fossil fuels in the years to come.

Research concerning lipid production from microalgae was stimulated in the past three decades by the increasing shortage of crude oil. Natural oil from microalgae seems to be the only renewable biofuel with the potential to completely displace petroleum-derived transport fuels because, at least in principle, it can be employed for the production of biodiesel in an economically effective and environmentally sustainable manner (Chisti, 2007). Although this point has been recently questioned by a life cycle analysis (LCA) (Clarens et al., 2010), the unavailability of industrial plant commercial data currently hinders any adequate LCA study, and more research efforts are needed not only to show the process feasibility at the industrial scale, but also to provide further experimental and theoretical support to the implied technology.

Microalgae are a highly diverse group of unicellular photosynthetic organisms comprising eukaryotes and prokaryotic cyanobacteria, that can grow at a much faster rate than plants thanks to their simple structure (Day et al., 1999). Unlike the case of first-generation biodiesel, the extensive cultivation of microalgae does not compete for agricultural land with food crops, since they can be grown on marginal land or in aquatic systems. However, in order to become economically feasible, the cultivation of microalgae for biofuel production requires high biomass and lipid productivity per area and minimum plant investment and operating costs.

The two options for massive production of microalgae are open systems, such as raceway ponds, and closed photobioreactors. Open cultures are inexpensive but bring about important drawbacks, including lower long-term productivity due to limited exposition to light, complex carbon management and large susceptibility to contamination (Wahal and Viamajala, 2010). In the case of photobioreactors, initial capital investments are certainly more demanding, but they can provide higher overall productivity thanks to better contaminant management and improved utilization of photosynthetically active radiation, carbon dioxide and other nutrients. The design of closed systems must be carefully optimized for each individual microalgal strains, according to its specific physiological and growth characteristics. Apparently, major technical and economic challenges still prevent the selection

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of an optimal reactor type at the commercial scale (Kunjapur and Eldridge, 2010).

Several researchers worldwide claim to possess technologies for the commercial production of biodiesel from microalgae, and many new companies have been recently developed (Singh and Gu, 2010; Zijffers et al., 2010; Grobbelaar, 2010). However, a number of technical challenges remain unsolved, including questions concerning large-scale microalgae recovery and oil separation processes. For these reasons, in spite of quite large scientific, technological and commercial interest, no industrial plants finalized to produce oil from microalgae are operated in the world (Singh and Gu, 2010): algae cultivation systems work well at the laboratory and small pilot/demonstration levels, but the process feasibility has not been demonstrated for large scale production yet.

One of the problems is that the best microalgae productivity achieved in pilot units does not exceed 10% of the maximum productivity calculated on the basis of sunlight irradiation and photosynthetic efficiency of microalgae (Rodolfi et al., 2009). In order to increase this value, it is first of all essential to understand the fundamental phenomena involved. At the biological/biochemical level, research is currently focused in increasing the lipid content and quality of microalgal strains (Chisti, 2007) also through genetic modifications (Courchesne et al., 2009). From the chemical engineering standpoint, parameters such as temperature, light regime, nutrient feed system, heat and mass transfer have to be finely tuned with substantial improvement of the photobioreactor set-up and operation. Of particular importance is also ensuring adequate supply of nutrients to avoid they can be growth-rate limiting factors.

Phototrophic microalgal production requires light, carbon dioxide, water and inorganic nutrients. Light is providing all energy for biomass accumulation and its absorption and conversion efficiency must be maximized in microalgal mass culture systems (Chisti, 2008; Carvalho et al., 2011). Another main issue to be addressed is carbon supply (Benemann et al., 1987; Oswald, 1988; Tapie and Bernard, 1988). Flue gases from power and chemical plants could represent an inexpensive CO_2 source (Chisti, 2007), but its concentration must be optimized avoiding both growth limitation by low CO_2 supply and also growth inhibition due to excess acidification (Ruiz-Marin et al., 2010). Besides carbon, nitrogen and phosphorus are the most important nutrients, for which a promising approach is using mineral salts contained in wastewaters from treatment plants (ammonium, nitrates and phosphates), thereby contributing also to the solution of water eutrophication problem (Ruiz-Marin et al., 2010; Chinnasamy et al., 2010). The combination of biofuel production from algae with CO_2 sequestration and wastewater bioremediation could result in an eco-friendly process and provide economical advantages to improve the still uneconomical large-scale second generation biofuel production system (Sheehan et al., 1998).

Although the molecular regulation of metabolic pathways in microalgae is not fully understood, in particularly at genomic and transcriptomic level, it is well known that their molecular composition is affected by nutrient availability in the growth medium (Guschina and Harwood, 2006; Ratledge and Wynn, 2002). In particular, cellular lipids content can be regulated by nutrient availability during growth (Rodolfi et al., 2009, Converti et al., 2009). Interestingly, nitrogen deficiency was shown to enhance lipid accumulation in different algal species (Oswald, 1988; Illman et al., 2000). However, a strong increase in lipids accumulated comes at the expense of a reduction in biomass growth, thus reducing overall productivity (Converti et al., 2009). In order to maximize both biomass growth and lipid synthesis, a two-phase strategy was successfully experimented: a first step with nutrient sufficient and high biomass production was followed by a step of nutrient depletion with lipids accumulation (Rodolfi et al., 2009).

The objective of our work is to access the effect of several parameters on microalgae growth and lipids accumulation, in order to better understand this process, and to give a contribution towards its industrialization and large-scale application. To this aim we focused on the marine species *Nannochloropsis salina* for its high biomass productivity and ability to accumulate large amounts of lipids (Boussiba et al., 1987). We evaluated growth performances and lipid content in this species in various culture conditions. In particular we focused on the effect of nitrogen content, CO_2 supply and concentration, and light/dark cycles. Finally, based on our experimental results, we summarized the parameters which could be more relevant in the design of large-scale photobioreactors for *N. salina* production.

2. Materials and methods

2.1. Microalgae and media composition

The microalgae species used was *N. salina* 40.85 (obtained from SAG-Goettingen), a marine species, which was cultured in sterilized sea salts (Sigma–Aldrich) 22 g/L solution enriched with f/2 Guillard solution (Sigma–Aldrich), as described by Guillard and Ryther (1962). For the experiments with different sources or concentration of nitrogen, the basic media was modified by changing nitrogen content and concentration (0.71 g/L of NH_4NO_3 (Sigma–Aldrich) and 1.2 g/L of $(NH_4)_2SO_4$ (Sigma–Aldrich) added to basic media for source of nitrogen experiments; 1.5 or 2 g/L of $NaNO_3$ (Sigma–Aldrich) added to basic media for optimization of growth experiments). Maintenance and propagation of culture were performed using the same medium added with 1% of Plant Agar (Duchefa Biochemie).

2.2. Growth analysis

Growth experiments were performed both in Erlenmeyer flasks (batch system) and in 0.25-L glass bottles under a continuous enriched CO_2 feed flow (semibatch system). Each autotrophic batch cultivation (250 mL) was carried out in duplicate. The medium and flasks were sterilized in an autoclave for 20 min at 121 °C in order to prevent any contamination. The growth temperature was 24 ± 1 °C, with artificial lighting (fluorescent tubes) under a continuous photon flux density of $120 \pm 10\ \mu E\ m^{-2}\ s^{-1}$, measured by a photoradiometer (LI-COR, Model LI-189). The duration of the experiment depended on the growth rate. Algal growth kinetics was measured by daily changes in optical density (measured at 750 nm, by a Perkin Elmer-Lambda Bio 40 spectrophotometer) and cells number. In the logarithmic growth phase cells number was related to optical density. For dry weight (DW) determinations cells were harvested with a 0.22 μm filter. DW was measured gravimetrically upon drying the filters at 100 °C for 4 h in a laboratory oven. The specific growth rate was calculated by the slope of logarithmic phase in terms of number of cells.

In order to study the effect of CO_2 concentration on growth, a number of experiments were conducted in the semibatch