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An experimental helical-tubular photobioreactor for continuous production of *Nannochloropsis* sp.

D. Briassoulis [a,*], P. Panagakis [a], M. Chionidis [a], D. Tzenos [b], A. Lalos [b], C. Tsinos [b], K. Berberidis [b], A. Jacobsen [c]

[a] Agricultural University of Athens, Agricultural Engineering Department, 11855 Athens, Greece
[b] University of Patras, Department of Computer Engineering and Informatics, RACTI RU-8, 26500 Patras, Greece
[c] University of Bergen, Department of Biology, P.O. Box 7803, N-5020 Bergen, Norway

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ABSTRACT

An experimental helical-tubular photobioreactor has been designed for controlled, continuous production of *Nannochloropsis* sp. Its main advantages are: (1) combination of large ratio of culture volume to surface area along with the optimised light penetration depth, (2) easy control of temperature and contaminants, (3) effective spatial distribution of fresh air and CO_2, (4) better CO_2 transfer through extensive interface surface between fresh air and culture-liquid medium and (5) novel automated flow-through sensor providing continuous cell concentration monitoring. *Nannochloropsis* sp. population density reached maximum value under rather high temperatures and combined natural and artificial light conditions. An average daily increase of 30×10^6 cells ml^{-1} was obtained at population densities above 350×10^6 cells ml^{-1} allowing daily harvesting rates of at least 10% the total volume. Measured cellular density productivity data and estimated volumetric productivity range of 1.10–3.03 g l^{-1} day^{-1}, are among the highest *Nannochloropsis* sp. productivities reported in the literature.

1. Introduction

Nannochloropsis sp. (Eustigmatophyceae) is cultivated in marine fish hatcheries as feed for rotifers and to create a 'green-water effect' in the larvae tanks (Lubzens et al., 1995). Continuous (vs. batch) production of micro-algae requires the adoption of specially designed fully closed and controlled photobioreactors such as: flat-plate systems (Cheng-Wu et al., 2001; Richmond and Cheng-Wu, 2001), tubular systems (Pulz, 2001) and coil-type systems (Watanabe et al., 1995).

Tubular systems are the most widely used commercial systems. Usually they are made of polypropylene acrylic or polyvinylchloride pipes having small internal diameters. Mixing and agitation of the culture is maintained by an air-pump forming bubbles. These systems typically use artificial light but there are also designs based on natural light. There are several types among which the 'Biofence' (Pulz, 2001), a complete system for large-scale production of micro-algae and photosynthetic bacteria. The main disadvantages of the tubular systems, varying however among the individual systems, concern the relatively high space requirements, high light energy requirements, cleaning problems and low efficiency in terms of mass production per unit of space. The hydrodynamic stress on the algae may vary, depending on the flow characteristics of each system (e.g. turbulent flow, pump type), from low to high. Likewise, the gas transfer to the culture may vary from low to high, depending on the flow characteristics and the air supply technique adopted. The scale up of these systems is reasonable. Their operational difficulties may also include: growth of algae in tube walls blocking light; high oxygen concentration that can inhibit photosynthesis; limit on the length of the tube in single run (Moholkar, 2008).

Flat-plate systems are also developed for the production of algae (Evens et al., 2000). Light is evenly emitted from a flat surface screen or from lamps above the culture. The plate surface is usually made of glass or optical light film and the circulation is achieved by the same means as with the tubular systems. These systems may also experience problems with relatively high space requirements, high light energy requirements, cleaning problems and possibly low efficiency in terms of mass production per unit of space (depending on the spacing requirements between the panels and the areal productivity constraint for outdoors application; crucial factors for systems indoors include: distance of light sources from panels, and temperature effect, illumination of one or both panel sides, light-path etc.). Their scale up potential seems to be difficult. Biomass output may be limited by photo-inhibition and problems have been reported with temperature control (Moholkar, 2008). The hydrodynamic stress on algae may vary from low to high.

The coil-type systems were mainly developed to improve on space utilisation as compared to the other categories. Among the

* Corresponding author. Tel.: +30 210 5294011; fax: +30 210 5294023.
E-mail address: briassou@aua.gr (D. Briassoulis).

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most important advantages (Watanabe et al., 1995), common to most coil-type systems (e.g. Biotechna Ltd., 1987 patent), are the larger ratio of surface area to culture volume to receive illumination effectively and the easy control of temperature and contaminants. Still the cleaning problems are not easy to solve while the hydrodynamic stress on algae and the gas transfer may vary from low to high. The scale up of these systems is easy. The operational difficulties observed with the tubular systems also apply in the coil systems: growth of algae in tube walls blocking light; high oxygen concentration that can inhibit photosynthesis; limit on the length of the tube in single run (Moholkar, 2008).

The existing systems represent a significant progress made so far, in meeting the massive continuous algae production needs. However, production of micro-algae in hatcheries is costly and often laborious. The few systems designed that are automated and closed cannot produce the large quantities of micro-algae required by the hatcheries. The main reason is that these systems are still lacking the efficiency (Ugwu et al., 2008) that would allow them to become optimal for use in hatcheries. Some of the key issues that could be improved include:

(a) Automated control and harvesting incorporated as an integral component in a closed system. This could improve significantly the cost efficiency of the system by reducing the labour needs and optimising the harvesting efficiency and control of inputs in the bioreactor, reduce contamination and control optimal conditions for the media. The development of an automated harvesting system relies heavily on the performance of an appropriately designed optical density (OD) sensor. In particular, a special OD sensor has to be developed and trained to the specific density and colour characteristics of the algae production under consideration, as no such tailored designed sensors are available in the marked.
(b) For a continuous massive algae production system to meet the needs of a hatchery and be operational and cost efficient, the bioreactor system design needs to be optimised with respect to several parameters in an integrated way: surface exposed to outdoors light; production volume per unit of space; air CO_2 to liquid media inter-phase; automated control and harvesting.

The main concept behind the present work is that an efficient system should be based on an integrated design approach and should be fully automated. It should be optimised with respect to key design parameters in an integrated way and also be automated in terms of controlling and harvesting operations and equipped with a well trained for the application case OD sensor.

This paper presents the optimised design of an experimental helical-tubular photobioreactor for continuous automated production of *Nannochloropsis* sp. and its performance characteristics under real outdoors conditions. This system was designed as a part of developing computerised continuous algae production (CAP) systems (ALFA, 2007).

2. Methods

2.1. Design concept

The design of the present experimental photobioreactor system is based on the concept of a coil-type bioreactor. An integrated two-phase (culture-liquid phase and air phase) helical-tubular photobioreactor suitable for continuous automated production of *Nannochloropsis* sp. under natural light (outdoors conditions), in combination with artificial light (continuous lighting), was designed. Its design was based on the following basic principles (technical details are given in the next section):

- Achieve full exploitation of solar radiation for maximum microalgae production especially in, but not limited to, South European regions; this required special design for operating under both, high temperatures during the spring-fall period and possible heating requirements during winter production. The final full-scale design is using efficiently available underground water to control temperature without any extra heating/cooling requirements. This is achieved at no cost as underground water is used regularly in most hatcheries operations in south Europe.
- Develop a cheap and simple system with optimised design parameters, including the two-phase systems, materials, energy and sensors-control systems
- Make optimal use of space, without sacrificing the exposed to the sunlight area, through the geometric characteristics of the helical-coil design and the materials selection, allowing simultaneously natural ventilation and diffuse light through openings between the coil spirals.
- Design a modular and versatile helical-coil photobioreactor system that may be adapted to include artificial light depending on the location/application
- Develop an automated novel flow-through sensor providing real-time continuous cell concentration monitoring and allowing fully automated harvesting.

The main parameters of the present experimental system optimised through the design of the helical-tubular coil-type system include: (1) combination of (a) a large ratio of culture volume over surface area (approximately 40 l m^{-2}; 196 l per 5 m^2 footprint) and (b) optimised light-path (all culture cells were exposed to light); the aim of this combination was for the culture to receive direct and/or diffuse illumination effectively from all directions, thereby increasing the incident light energy input per unit volume of culture and reducing the self-shadowing phenomena; (2) easy control of temperature through the exploitation of underground water of constant temperature; (3) avoidance of contamination being a closed bioreactor (a general feature of coil-type systems); (4) provision of more effective spatial interface of the two-phase system; the aim here was to allow for a better transfer of fresh air plus additional CO_2 gas in the culture through its injection via multiple outlet points; the outlets were distributed inside the whole length of the pipe, resulting in a continuous inter-phase between the two phases, a better mixing and up-taking of nutrients and CO_2; (5) efficient removal of oxygen produced in the light phase by keeping short the length of each modular helical-tubular coil so as to avoid oxygen saturation; (6) operation under full daylight conditions without providing any dark phase in the pathway of the media; (7) complimentary artificial light system during night-time and overcast periods without any time interval of dark phase throughout the production period; (8) control of pH level by automatically adjusting the supply of CO_2 along the whole length of the helical-tubular coil through the continuous air phase-media inter-phase; (9) use of specific type of media circulating pump to avoid cell damage due to hydrodynamic stress; (10) advanced harvesting system based on automatic measurement of culture density by means of an innovative flow-through sensor providing continuous cell concentration monitoring.

2.2. Design requirements for Nannochloropsis sp

Based on extensive literature review and results from laboratory and outdoors pilot tests (ALFA, 2007) the most critical design requirements for growing *Nannochloropsis* sp. are summarised below.

2.2.1. Temperature

Most commonly cultured species of micro-algae tolerate temperatures between 16 and 27 °C, depending on the culture medium composition, the species and strain cultured (Coutteau, 1996). Temperatures lower than 16 °C will slow down growth, whereas those higher than 35 °C are lethal for a number of species (FAO, 1996); not for the mesophilic micro-algae though. According to the results of several laboratory experiments the highest *Nannochloropsis* sp. growth rate may be achieved at the suggested optimum temperature ranges: 15–20 °C (James et al., 1989); 22 ± 2 °C (Brown et al.,1993); 25 ± 1 °C (Yamasaki and Hirata, 1995); 19–21 °C and 24–26 °C (Abu-Rezq et al., 1999); 20–30 °C (Oellermann, 2001) and 25–29 °C for *Nannochloropsis oceanica* (Sandnes et al., 2005). Accordingly, the maximum design temperature for the experimental culture was set initially at 30 °C. However, preliminary laboratory tests carried out in the framework of the present work for *Nannochloropsis* sp. did not identify a maximum critical temperature up to 40 °C.

2.2.2. pH level and CO_2

For most cultured algal species pH range is 7–9, with the optimum range being 8.2–8.7 (Coutteau, 1996). A complete culture collapse can result from a failure to maintain an acceptable pH due to the disruption of many cellular processes. To obtain high densities of *Nannochloropsis* sp. CO_2 should be supplied. Hu and Gao (2003) showed that elevation of CO_2 from 350 to 2800 μl l^{-1} raised biomass yield by 39% in photoautotrophic culture and 21% in mixotrophic culture. The design pH range for the experimental culture was set initially at 7–9. Laboratory tests of the present work for *Nannochloropsis* sp. confirmed a low limit of 6.5 and an upper limit of 10.

2.2.3. Salinity

Most species grow best at salinities of 20–24‰ slightly lower than that of their native habitat (Coutteau, 1996). For *Nannochloropsis* sp. various salinities have been proposed: 35‰ (Brown et al., 1993), 20–40‰ (Abu-Rezq et al., 1999) and 0–36‰ (Oellermann, 2001). Based on the above, the salinity for the experimental culture was set at 35‰.

2.2.4. Light requirements

Recommended range of light intensity for growing micro-algae in hatcheries is 2500–8000 lux (Oellermann, 2001); equivalent to 40–160 μmol photons m^{-2} s^{-1} (PAR) (CSIRO, 2005). Another range of recommended values of light intensity for growing micro-algae is 2500–5000 lux (40–100 μmol photons m^{-2} s^{-1} (PAR)) (FAO, 1996). In outdoor reactors with a long light-path of ca 10 cm, 1800–2100 μmol photons m^{-2} s^{-1} (PAR; for 5 h at midday) is found to be optimal for *Nannochloropsis* sp. (Richmond and Cheng-Wu, 2001; Zou and Richmond, 1999). Maximum daily productivity for *N. oceanica* cultivated in a bio-fence system occurs at 1030 μmol photons m^{-2} s^{-1} average daily radiation (natural 111 μmol photons m^{-2} s^{-1} plus artificial 921 μmol photons m^{-2} s^{-1}) in climate-regulated greenhouses (Sandnes et al., 2005).

2.2.5. Flow characteristics

Growth rates of some micro-algae increase initially with increasing turbulence however the growth decreases sharply with further increase of the gas velocity due to cell damage (Merchuk et al., 2000); small-scale turbulence may be beneficial by increasing diffusion rate of nutrients to the cell surface (Lazier and Mann, 1989); negative effects may occur through mechanical damage, behavioural alteration (Karp-Boss et al., 2000) and physiological impairment (Estrada and Berdalet, 1997). *Nannochloropsis* sp. is found to be quite resistant to turbulence (with blue–green algae being the most resistant and dinoflagellates being most sensitive strains; Thomas and Gibson, 1990). Nevertheless, it was decided that a specific type of media circulating pump should be used with the proposed photobioreactor to avoid possible hydrodynamic stress and thus, remove one extra source of uncertainty (Chisti, 1999).

2.2.6. Aeration

Mixing is necessary to prevent sedimentation of the algae, ensure that all cells are equally exposed to light and nutrients, avoid thermal stratification and improve gas exchange between the culture medium and the air. Pure CO_2 may also be supplemented to air supply (Coutteau, 1996). Major importance was placed in the design of the experimental helical-coil photobioreactor on meeting the aeration needs to the highest possible degree and on minimising the need (and cost) for supplementing pure CO_2.

2.2.7. Nutrients

The Guillard's f2 medium has been used extensively and is suitable for the indoors or outdoors cultivation and growth of *Nannochloropsis* sp. (Chini Zittelli et al., 1999, 2003; Coutteau, 1996). The f2 medium feeding system was incorporated in the automated control/harvesting of the helical-coil photobioreactor. The f2 medium quantities supplied, following preliminary laboratory investigation, are shown in Table 1.

2.2.8. Supplementary artificial light requirements for Nannochloropsis sp

Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as being the active portions for photosynthesis (Coutteau, 1996) with a minimum duration of illumination of 18 h day^{-1}. Preliminary experimental investigation was carried out in the laboratory on the performance of four different categories of lamps. LED, fluorescent, high pressure sodium and metal halide lamps were tested with respect to algae growth for the same light intensity and temperature. The experiments led to the conclusion that the best choice for an artificial light source during the night periods were the fluorescent cool daylight lamps (colour: 6500 K; efficacy: 89–104 lm W^{-1}) as they resulted in the most stable production (lab conditions). It was shown that *Nannochloropsis* sp. needs more blue than red light (probably an acclimatisation effect in the aquatic environment, where water absorbs stronger the red than the blue light).

2.3. Experimental helical-tubular pilot-scale photobioreactor design

Three experimental pilot-scale trials (I, II, III) were performed at the Agricultural University of Athens facilities (lat. 37°58′N, long. 23°32′E) under various experimental conditions (Table 1). The initial design parameters were set as described in the previous section. Based on the operational and technical problems encountered and the evaluation of the performance results obtained during each trial, modifications, improvements or adjustments were made to optimize the design of the various pilot-scale photobioreactor components and finalise the key design parameters. The optimised parameters were used subsequently, and integrated with the automated controlling and harvesting system, to design, construct and test an experimental full-scale modular unit at the facilities of a commercial hatchery unit. The full-scale experimental unit design is described in Section 2.6.

A flexible food-contact quality (non-toxic) transparent pipe (100 mm internal diameter; 5 mm thickness; 25 m length; 196 l volume) made of PVC and reinforced with steel spiral was used to create the photobioreactor coil. The light-path of the coil was 10 cm. The flexible pipe looped around the South oriented core frame structure in a helical-conical coil formation (maximum exposure to sun light during winter time; constant slope of

Table 1
Experimental conditions during the three experimental Trials.

Experimental parameters*	Trial I May 09 – July 04, 2006	Trial II November 2006	Trial III December 2006 – January 2007	Up-scaled experiment July 1 – August 7, 2007
Initial cell density (cells ml^{-1})	12.2×10^6	55.2×10^6	17.4×10^6	122.8×10^6
Solar PAR irradiannce				
($\mu mol\ m^{-2}\ s^{-1}$) daily average	618.97	267.77	176.01	529.43
($\mu mol\ m^{-2}\ s^{-1}$) maximum	2364.12	1365.72	1177.72	2154.09
Diffuse PAR irradiannce				
($\mu mol\ m^{-2}\ s^{-1}$) daily average	194.88	86.21	51.41	172.27
($\mu mol\ m^{-2}\ s^{-1}$) maximum	703.09	327.95	195.58	641.79
CO_2 supplying	Manual checking (only when pH exceeded 9.0)	Automatically regulated by a sensor	Automatically regulated by a sensor	Automatically regulated by a sensor
pH range	6.5–10.0**	7.3–9.2	8.4–9.5	6.8 – 8.6
Ambient temperatures (°C)	24.0 (avg) 12.9–35.2 (range)	15.0 (avg) 8.9–22.3 (range)	7.3 (avg) 0.8–17.8 (range)	36.0 (avg) 20.0–45.0 (range)
Daily feeding schedule*** (f2); ($g\ l^{-1}$)	0.5	0.5	0.25	0.15
Complementary lighting (fluorescent lamps)	Four 58 W (during the night)	Six 58 W (when overcast and during night)	Six 58 W (when overcast and during night)	4 lamps 58 W for 24 h (core of tank) 12 lamps 58 W for 12 h, night (core of coil)

* Variable for testing purposes.
** CO_2 supply failure.
*** Feeding was performed during the day.

1:14). The pipe diameter combined with the helix geometry were designed in a way to allow for the direct and diffuse sunlight to penetrate the media mass flowing along the whole length of the coil. In addition, the design allowed air to flow in, between the coil spirals, offering natural ventilation conditions to the system. The helix was designed so as to induce unobstructed flow of the air released inside the culture media simultaneously and continuously along the whole length of the coil to the top of the tube, allowing it to escape through a special degassing device and prevent formation of air pockets during the circulation of the algae. Continuous supply of air (which is also the main supply of CO_2) into the culture was achieved via a perforated flexible transparent 12 mm PVC pipe inserted into the coil at its lower point and ending up at the top with its end closed. Air was introduced by means of an air-pump (flow rate 120 l min^{-1}; static pressure 29.4 kPa).

The helical-coil was connected to a transparent 70 l main tank (30 cm diameter), with the culture entering the upper part of the tank free of air and getting out from the bottom. Additional fresh air was supplied in the tank through another set of perforated pipes. The upper part of the main tank was placed at the highest level of the system so as to act as a buffer for the two phases (water and air) and for degassing, and simultaneously to serve as an operational unit (i.e. supporting sensors for temperature, pH monitoring, nutrients feeding, etc.).

The circulation pump (rotary vane type; 1.5 kW power; 6000 l h^{-1} volumetric flow; three phase motor rotating at a speed of 820 rev min^{-1}) was chosen based on the need to avoid possible cell damage associated with mechanical pumping and the requirement to be resistant to aggressive chemicals as hypochlorite, which was mainly used for the sterilization. It was placed between the tank and the air adaptor. The culture circulated at a design speed of 0.21 m s^{-1} (Reynolds number approximately 21,200 – turbulent flow).

No dark phase was provided to the culture by this system in all three trials, based on the results of preliminary laboratory experiments on the effect of light on the growth of the culture. Six cool daylight fluorescent lamps (58 W each) were placed inside the core frame structure. They operated on a timer according to season (e.g. duration of natural light) and when overcast.

2.4. Pilot-scale experiments

Three pilot-scale experiments were conducted in the period of 9 May – 4 July 2006 (Trial I), 7–28 November (Trial II) and from 11 December 2006 to 4 January 2007 (Trial III). In all three pilot-scale experiments 35 l of *Nannochloropsis* sp. culture and 175 l of 0.2 micron filtered artificial salt-water (salinity 35‰) were used to start-up the system. One day before the start-up of each trial the system was cleaned thoroughly using a mixture of fresh water and hypochlorite solution and thereby neutralized. Cell density and pH were measured three times daily (09:00, 12:00 and 15:00 h). Cells were counted during the pilot trials using 0.1-mm-deep Neubauer haemacytometer by a light microscope (×40 magnification), whereas a portable and a desktop pH measuring device, were used to measure the pH. To ensure reliability of measurements, cell density was measured independently by three different trained researchers each time. In the case of the full-scale experiments measurements were performed by both, staff of the hatchery and members of the research team.

Trial I run under high summer temperatures and long daylight duration (Table 1), so drip cooling (5–10 l water min^{-1}) using well water was utilized to reduce the temperature of the culture. During Trial II an R22 chiller (power 1.5 kW; COP ≈ 3; cooling capacity 4.5 kW) was used for cooling, whereas during Trial III the heating demand was covered by means of four 300 W resistors. It should be noted that for the experimental full-scale trial during summer period cooling was achieved efficiently through a simple spaying system by using natural underground water. The cooling/heating systems used for the pilot trials were designed to serve only the needs of the pilot experiments and do not represent an integral part of the system design.

2.5. Automated flow-through density sensor and harvesting system

Parallel to the photobioreactor design, a fully automated harvesting system was also designed and constructed (Fig. 1). The main principle of the system was based on a novel automated flow-through sensor which provides continuous cell concentration monitoring. A market search carried out systematically through

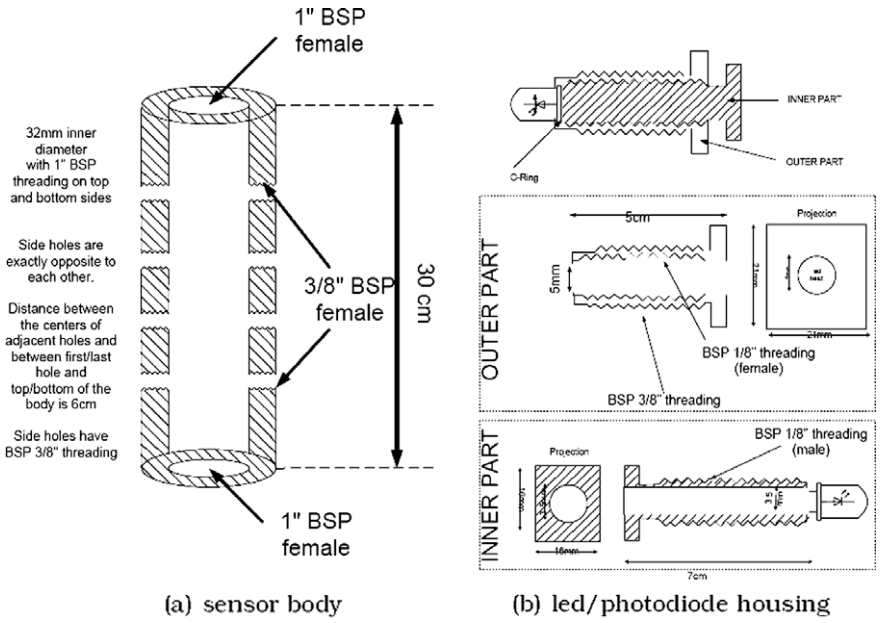

Fig. 1. Sensor body and LED photodiode housing.

the internet and representatives of several companies showed that most of the commercially available sensors have been designed for outdoors measurements in water environments and their specifications did not meet the needs of the harvesting system for the particular photobioreactor design. There are a few commercial sensors which are able to measure the high cell concentration values encountered in the restricted environment of the bioreactor's main tank. They have a relatively high cost though. Thus, in order to keep the cost of the overall system low, a new sensor was designed, implemented and tested. This sensor was designed to meet the technical requirements of the specific application. The sensor is able to provide very close approximations of the *Nannochloropsis* sp. algae concentrations within the range of interest for the given photobioreactor. An additional feature of the proposed design is that it can be used for measurements in a wider field of algae species as well as for other tasks that are common in a fish hatchery unit (e.g. measurement of algae concentration in a solution which also includes rotifers and their waste). Moreover, the proposed sensor is highly reconfigurable and easy to calibrate. The measurements are subject to sophisticated processing via the use of a properly designed neural network. An in-depth analysis of the performance and sensitivity of the designed sensor is beyond the scope of this paper.

It is often assumed that in a photobioreactor system, such as the helical-coil system, the dominant factor causing turbidity in the tank is the concentration of the live algal cells. This assumption might not be always realistic. For example, the presence of dead cells or a possible contamination in the culture, are factors that may also affect the turbidity. For this reason, it would be desirable to have some kind of diversity in the collected data, so that, during a processing phase, the noisy effect of these factors is significantly reduced (i.e. filtered out). Such a diversity can be obtained either by measuring the effect of the same light source with multiple photosensors positioned at different locations within the media, or by making many independent simultaneous measurements (using different light sources and/or photosensor types) on the same sample, or by a proper combination of both strategies.

The operation of the designed sensor system is similar to that of a turbidity meter. That is, a number of light sources emit beams of light through the algae mass directly into the photosensors which in turn read the response. In the absence of reflection losses, the intensity of a light beam passing through a dielectric sample is reduced, mainly due to absorption and scattering. The reduction in transmitted light intensity due to scattering is related to the sample's turbidity. The Beer–Lambert law (Eq. (1)) describes the effects of both absorption and turbidity on the transmitted light power (Bohren and Huffman, 1983):

$$P_T = P_0 e^{-(\alpha+\tau)l}, \tag{1}$$

where P_T is the power of the light transmitted through the sample, P_0 is the power of the light incident on the sample, α is the absorption coefficient per unit length, τ is the turbidity per unit length and l is the length of the light-path in the sample.

The designed sensor employs four different monochromatic LEDs whose particular spectral characteristics (i.e. blue at 470 nm, green at 518 nm, red at 630 nm and infrared at 940 nm) were decided after extensive laboratory experimentation using several combinations of LEDs with different spectral characteristics on a wide range of *Nannochloropsis* sp. algae concentrations.

The sensor provides samples of four different measurements forming a 4×1 vector:

$$S(c) = [B(c), G(c), R(c), IR(c)], \tag{2}$$

where $B(C)$, $G(C)$, $R(C)$, $IR(C)$ are the output voltages of the photodiodes which are placed opposite to the blue, green, red and infrared LEDs, respectively, and C is the concentration of the sample inside the sensor. The objective is to obtain the estimation of C by using the above 4 measurements. This is a pattern recognition problem that can be solved via the use of a proper neural network (NN). A NN is a massively parallel distributed processor made up of simple processing units, which has a natural trait of storing empirical knowledge and making it available for use. It resembles the brain in two aspects: (1) Knowledge is acquired by the network from its environment through a training process and (2) Interneuron connection strengths, known as synaptic weights, are used to store the acquired knowledge. The procedure used to perform the training process is called a training algorithm, the function of which is to modify the synaptic weights of the network in an orderly fashion to attain a desired design objective.

The LEDs responses were expected to be associated to exponential processes with different parameters thus providing the desired diversity to the measurements. This fact has been verified via a series of experiments carried out in the laboratory by using algae paste of *Nannochloropsis* sp (see Fig. 2). The experiments started with a high density of 400×10^6 cells ml^{-1}. The actual density value was determined by manually counting the algae cells in a microscope using a 0.1-mm-deep Neubauer haemacytometer. The sample was filled into the sensor. Then, the sample was diluted by a percentage of α%, thus reducing the concentration of the algae also by α%. The diluted sample was inserted into the sensor and the measurements were repeated. The whole procedure was repeated until a low enough concentration was reached. The neural network was trained in this way for several concentrations and then tested and confirmed with a number of samples density measurement experiments.

Note that photodiodes (for the visible spectrum) and phototransistors (for the infrared beam) were used as photosensors, because they tend to vary their response in a linear way with respect to the power of the excitation beam. The sensor is typically installed in a continuous algae production unit, cascaded to the circulation flow. As the algae fluid media flows through the sensor's body, it is intercepted by light beams emitted at different spectra by the corresponding LEDs, and the response is read through the photosensors which are located opposite to each LED, in pairs. The whole process was performed by a properly designed driving circuit board, which also ensures that the LEDs emit always light of exactly the same power. A specially equipped computer system controlled the electronic board, by running the appropriate custom-made software. Each measurement taken from the sensor resulted in a 4×1 vector of voltages, which was fed to the Signal Processing Unit, also implemented in software. The output of this unit is an estimation of the algal concentration of the media that is flowing through the sensor at the time of the measurement. It should be noted that the Signal Processing Unit is re-trainable. Training might be required in order to improve the sensor's performance (i.e. reduce the estimation error). Also, new training must be performed in order to support new species of algae. However, after the Signal Processing Unit is appropriately trained, the sensor's operation is completely automated. Preliminary results in the laboratory had shown that sufficiently low estimation errors are achievable, provided that the training dataset is large enough and representative of the typical conditions of the medium monitored.

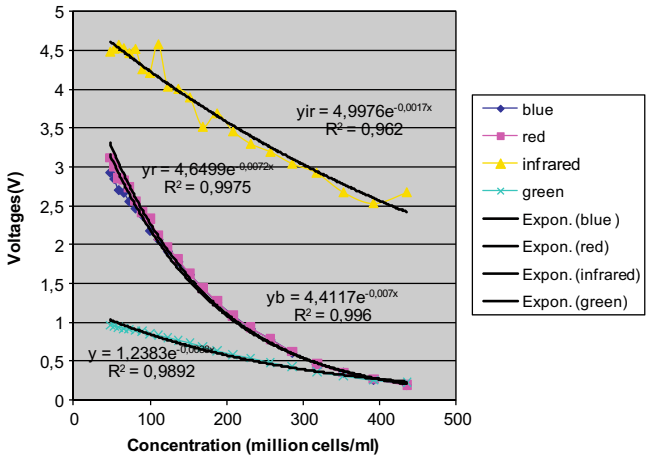

Fig. 2. Exponential curves that fit the response of the photodiodes at the different light beams (red, blue, green, infrared). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

At this point it should be noted that, almost simultaneously with the development of the proposed sensor, a similar sensor design was presented in Sandnes et al. (2006). The authors in Sandnes et al. (2006) used infrared LEDs (880 nm) exclusively, since the light absorption of the *Nannocloropsis* algae species decreases sharply to a minimum value within the near infrared (NIR) range. This is due to the lack of chlorophyll *b* and *c* pigments in this algae species. The exponential curves describing the relationship between the output voltage of the photodiodes and the algae concentration are depicted in Fig. 2 for every monochromatic LED in the proposed design. It is obvious that the infrared LED is the most suitable for the concentration estimation since it exhibits the best relationship between the concentration and the ranges of the corresponding output voltage. However, it was chosen to design a sensor comprising all the suggested four different monochromatic LEDs in order to make it highly reconfigurable and able to be applied in a wider range of applications.

The sensor presented in Sandnes et al. (2006) is suitable for the *Nannocloropsis* case. However, different species appear to have different behaviour in the absorption of light, thus calibration is needed every time the species of the cultivation is changed. Moreover it is possible that calibration of the sensor is also needed at the different stages of a cultivation of a specific species as variations in the light absorption may appear. For example, some pigments can evolve during growth as a function of illumination conditions. The proposed sensor provides the flexibility of a full-software calibration by simply applying a re-training procedure of the neural network. The proposed sensor also may be used for monitoring and control of other production sub-units of a fish hatchery unit. For example, one possible use could be the measurement of the algae concentration in a tank with rotifers. Rotifers consume the algae and produce waste resulting in a solution with a mixture of elements that have very different light absorption behaviour than a pure algae solution. The proposed generic sensor with an appropriate training phase can be used successfully for this application as well.

The reasons a neural network was chosen for the data processing stage is that neural nets, in general, are powerful tools that can be used to solve a broad range of problems due to their high adaptability. They use optimally the inputs (in the present case, the photodiodes output voltages), so if an input appears to have less contribution it is appropriately weighted in the internal computations. From a pattern recognition point of view, it is preferable to incorporate inputs with less contribution as well (i.e., green, blue and red) and then leave the involved learning mechanism (the neural network in this case) to handle appropriately each input. Finally, neural networks are capable of simulating and incorporating in their functionality non-desirable phenomena that may appear during the running of the system such as variations in the light intensity due to the temperature of the LEDs or because they wear out, the effects of the medium temperature on the measurements etc.

In order to verify the functionality of the designed sensor the absolute error of the density estimation within the interval 5 to 145×10^6 cells ml^{-1} was calculated. The results were obtained through training of the neural network with samples obtained from the up-scaled experimental photobioreactor system installed at the facilities of a hatchery unit (Agrosaronikos S.A.) in Salamina island Greece. The absolute estimation error at the densities of interest was found to be always below 8×10^6 cells ml^{-1} (maximum error estimated at 9%). No particular dependence of the calculated relative error on the value of the measured concentration was observed for the densities interval of 5 to 145×10^6 cells ml^{-1}. Thus, it is expected that in higher concentrations the relative error will remain bounded within the same range as in low to medium concentrations. This claim has been verified through extensive laboratory tests, some of which are shown in Fig. 2.

Having estimated the concentration values, the system sequentially activates a series of electro-valves and peristaltic pumps, depending on the population density value. The production distribution can be achieved in various alternative ways, depending on the particular design of the crops feeding and tanks greening systems and the available packaging options for the excess production. Two example cases could be: (a) the electro-valves and the peristaltic pumps allow harvesting of a specific algae quantity to a storage tank. This storage tank maintains the daily algae demand plus a potential overproduction and acts as the main buffer from which algae are further distributed to other consumption sub-systems (i.e. rotifers, larvae, etc.) via a dosometric pump and (b) A secondary pump allows continuous circulation of the culture from the storage tank to the photobioreactor via a loop made of flexible PVC pipes. Activation of electro-valves placed along the loop over each consumption sub-system provides the specific daily algae demand.

2.6. Up-scaled experimental system

The results of the three pilot trials and the sensor development were used to optimize the system through the up-scaled experimental design (in terms of the selected ranges of critical parameters and by introducing automated monitoring of pH, CO_2 supply and temperature control, automated harvesting based on estimated cell density, artificial lighting etc.). The 588 l up-scaled experimental system was composed of 363 l coil PVC tubes and the 225 l external 13 cm wide, annular section of the tank (Fig. 3). The tank, made of Plexiglas, did not operate as a conventional buffer tank, but as a hollow tube with a light-path of ca 13 cm as only the external annular section of it was used for the algae circulation. The internal sections of the tank were used for artificial lighting and cooling. The system was installed at the facilities of Agrosaronikos S.A. The scaled-up experimental unit coil consisted of four parallel helicoidally arranged tubes each having an internal diameter of 76 mm (changed from 100 cm down to 76 cm for technical reasons by the manufacturer of the system; initially designed with a diameter of 100 mm). The four parallel tubes were used to create the coil though manifolds to allow for a steady supply of air along the whole length of each tube by means of internal perforated small diameter tubes, following the experience gained by the pilot trials. Using longer tubes would not allow a steady supply of air supply along the whole length of the tubes. The pump used was a 900 rpm, rotary vane type pump with flexible impeller having an approximate volumetric flow of 20 $m^3\ h^{-1}$. This system operated during summer time (June 26 – August 8, 2007) under high culture temperatures (22.9–31.2 °C) and with a daylight duration ranging between 14:48 h (June 26) and 13:57 h (August 8) (Table 1). This system fully exploited the very high daily average PAR solar irradiance 530 μmol photons $m^{-2}\ s^{-1}$ (Table 1) to which the part of the coil (light-path 7.5 cm) and tank (light-path 13 cm) oriented to the south, was exposed to. All other parts of the coil and tank, including the internal core sides, were exposed to a relatively high daily average diffuse PAR irradiance of 172 μmol photons $m^{-2}\ s^{-1}$ (Table 1). Artificial lights were used (Fig. 3) in a specially formed internal core within the tank (24 h) and inside the interior space of the coil during night, so that no dark phase was provided (Table 1). The air supplying perforated pipes were distributed along the whole length of the coil, inside the coil tubes, while additional air was supplied to the main tank (Fig. 3). The pH values varied between 8.3 and 8.6 controlled by means of additional CO_2 supplied through the air phase of the system. The total volumetric air flow was approximately 600 l min^{-1}. The culture temperature was kept between 23.4 and 28.3 °C using underground water with a constant temperature of 21 °C. In particular, a sprinkler system spraying the helical-coil was used in combination with a cooling system designed in the form of an internal core of the main tank (using the subsaline underground water Fig. 3).

Fig. 3. Up-scaled experimental photobioreactor system in operation.

3. Results and discussion

3.1. Pilot-scale experiments

The initial cell density in the *Nannochloropsis* sp. culture in Trial I was12.2×10^6 cells ml^{-1} (May 9) and the measured initial pH was 8.2. On June 9 the population density reached a maximum of 596×10^6 cells ml^{-1} (Fig. 4a). Daily batch harvesting of 19 l (i.e. 9%) was initiated on May 22. For reasons of testing on May 22 and June 14, 42 and 54 l were harvested, respectively. The temperature in the culture exceeded the upper optimum limit of 26 °C (Abu-Rezq et al., 1999) for 37.1% of the time with a maximum of 39.8 °C, while it was recorded below the lower optimum limit at 17.4% of the time (19 °C; Abu-Rezq et al., 1999) and was only within the optimum range of 19–26 °C for 45.5% of the time (air temperatures in Table 1).

In Trial II the initial density of *Nannochloropsis* sp. was increased to 55.2×10^6 cells ml^{-1} for comparison purposes and the pH was 8.0 (November 7). Daily batch harvesting of 19 l (i.e. 9%) was initiated on November 21 and continued until November 25. On November 20 population reached a maximum density of 485.7×10^6 cells ml^{-1} (Fig. 4a). Culture temperature exceeded the upper optimum limit for 32.1% (maximum 33.4 °C) while it was lower than the lower optimum limit for 18.9% of the time (minimum 15.8 °C) and was within the optimum range for 49.0% of the time.

The initial cell density in Trial III was 17.4×10^6 cells ml^{-1} and the initial pH of 8.0. On December 30, the population reached a maximum density of 382.4×10^6 cells ml^{-1} (Fig. 4a). Unfortunately, contamination problems forced this experiment to a premature end. Culture temperature exceeded the upper optimum limit for 3.9% of the time (maximum 28.1 °C), was lower than the lower optimum limit for 40.6% of the time (minimum 13.9 °C) and was within the optimum range for 55.5% of the time.

The results presented in Fig. 4a revealed that at population density levels between 160 and 260×10^6 cells ml^{-1} the daily increase in cells numbers varied between 21 and 57×10^6 cells ml^{-1}, whereas at population density levels between 307 and 550×10^6 cells ml^{-1} the daily increase in cells numbers varied between 21 and 44×10^6 cells ml^{-1}. Although this variability may be attributed to the combined effect of several interacting factors, it

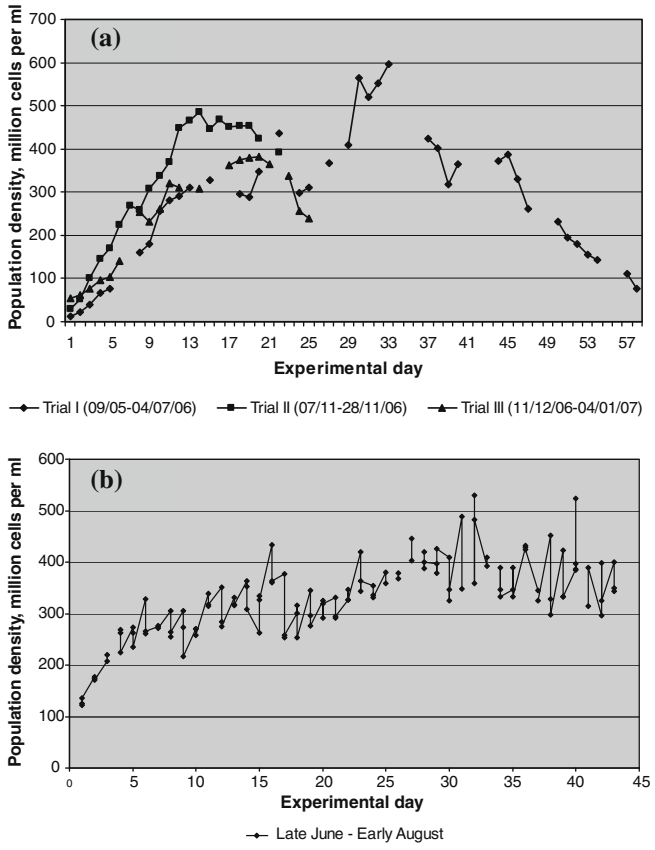

Fig. 4. (a) *Nannochloropsis* sp. population density during pilot-scale experiments. (b) *Nannochloropsis* sp. population density under up-scaled experimental production conditions.

may be concluded that an average daily increase in cells numbers of 30×10^6 cells ml^{-1} may be easily obtained by the helical-coil system operated outdoors at population densities above 350×10^6 cells ml^{-1} allowing harvesting rates of at least 10% of the total volume per day.

The results obtained through the pilot trials suggest that the variable initial density of the culture, the wide range of pH values allowed and the variable CO_2 supply to control this pH range, did not affect significantly the culture growth rate of the tested system. It was also shown that high temperatures do not really affect the high growth rates achieved by this system. Thus, it was considered adequate to control the temperatures of the optimised up-scaled system only within a rather wide range of temperatures by using underground water spraying.

3.2. Up-scaled experimental system

The productivity data obtained from the up-scaled experimental system, expressed as cell density, are shown in Fig. 4b. Daily harvesting (applied from July 1 to August 7) averaged 78.0 ± 4.4 l (13.3% of total volume per day) and had a mean cell density equal to $337.2 \pm 6.0 \times 10^6$ cells ml^{-1}. On June 29 population density reached a maximum value of 529.6×10^6 cells ml^{-1}, which is within the range of the maximum densities obtained with the pilot photobioreactor system during Trials I and II. The up-scaled system was in experimental operation only for a short period supporting the hatchery unit and providing additional data for possible revisions and future improvements of the system.

The dry weight of the production was not measured during these experiments. However presuming that each cell of *Nannochloropsis* sp. has a dry weight of approximately 6 pg (FAO, 1996) it is possible to estimate the daily volumetric productivity (g l^{-1} day^{-1}) as equal to 2.02 g l^{-1} day^{-1} (i.e. 337.2×10^9 cells l^{-1} $day^{-1} \times 6$ pg $cell^{-1}$). If another assumption, is made based on the measured data reported in the work of (Fábregas et al., 2004) that each *Nannochloropsis* sp. cell has a dry weight of approximately 3.26 pg then, the estimated daily volumetric productivity (g l^{-1} day^{-1}) is equal to 1.10 g l^{-1} day^{-1} (i.e. 337.2×10^9 cells l^{-1} $day^{-1} \times 3.26$ pg $cell^{-1}$). In another case, an industrial-size flat-plate glass reactor for mass production of *Nannochloropsis* sp. outdoors yielded a range of dry weight measurements of 3.60–4.80 pg for each *Nannochloropsis* sp. cell. Based on these data, the estimated daily volumetric productivity (g l^{-1} day^{-1}) of the present system is 1.21–1.63 g l^{-1} day^{-1}. The work of (Zou and Richmond, 1999) yielded a range of dry weight measurements of 6.00 (April–June) – 9.00 (July–September.) pg for each *Nannochloropsis* sp. cell. If these values are used as a reference, then the daily volumetric productivity (g l^{-1} day^{-1}) of the present system is estimated to be between 2.02 and 3.03 g l^{-1} day^{-1}. The above range was confirmed independently by CSIRO (2009), indicating that the ANACC cell dry weight data show a range of 3–9 pg per cell for different strains of *Nannochloropsis*.

3.3. Comparative evaluation of performance

For comparison reasons productivity data reported in the literature for various systems of photobioreactors are given in Table 2 and references therein. In the majority of these cases, concerning a variety of photobioreactor systems operating under natural or combined illumination conditions, the productivities range between 0.17 and 0.76 g l^{-1} day^{-1}. A rather high productivity has been reported for a fed-batch culture (Xu et al., 2004). In this case, feeding glucose solution, the biomass reached 1.1 g l^{-1} day^{-1} after 10 days' culture while the maximum of biomass (1.2 g l^{-1} day^{-1}) was obtained with the supplement of the mixture of glucose and nitrate solution. Some interesting results are also presented in Zou and Richmond (1999) based on the investigation of the effect of light-path length in a series of outdoor flat-plate reactors on the output rate of *Nannochloropsis* sp. cell mass. In this case there was a continuous supply of 1% CO_2 into the compressed air stream. The optimal volumetric productivity of 0.48 (g l^{-1} day^{-1}) was obtained for maximum areal productivity (22.5 dry weight g m^{-2} day^{-1}) at an optimum light-path for culturing *Nannochloropsis* sp. in vertical reactors ca 10 cm. Also, a maximum volumetric productivity of 1.70 (g l^{-1} day^{-1}) was obtained for minimum areal productivity (11.0 dry weight g m^{-2} day^{-1}) at a light-path of 2 cm resulting in less efficient use of radiation. According to (Zou and Richmond, 1999), for the slow growing *Nannochloropsis* sp. cells, the light regime prevailing in association with the more narrow light-paths could not be effectively used by *Nannochloropsis* sp. Analogous results were reported in Richmond and Cheng-Wu (2001) where the optimal population density (i.e. which results in the highest areal productivity) in the 10 cm plate reactor was obtained, yielding an annual average of ca. 240 mg l^{-1} day^{-1}. Higher values of 0.8 and 0.35 dry weight g l^{-1} day^{-1} were obtained in flat-plate photobioreactors with 1.3 and 5.2 cm of light-paths, respectively, associated however with lower efficiency of radiation (5.5 and 9.25 as compared to 12.1 g m^{-2} day^{-1}). A mean volumetric productivity of 0.61 g l^{-1} day^{-1}, increasing to 0.97 g l^{-1} day^{-1} was obtained by Chini Zittelli et al. (1999) with continuous illumination of 115 μmol photon m^{-2} s^{-1} on one side and on both sides of the panels, respectively. The higher productivity achieved when both sides were illuminated was accompanied with a lower efficiency of light conversion though (from 0.80 g mol $photon^{-1}$ for one side illumination down to 0.64 g mol $photon^{-1}$). In a recent work (Rodolfi et al., 2009) *Nannochloropsis* sp. was grown outdoors in 110 l Green Wall Panel photobioreactors under nutrient sufficient and deficient

Table 2
Productivity of *Nannochloropsis* sp. reported in the technical literature for some photobioreactors.

Photobioreactor	Volume (l)	Productivity ($g\,l^{-1}\,day^{-1}$)	Experimental period	Illumination	References
One-tube near horizontal tubular	10.2	0.73 ± 0.37 0.76 ± 0.39	9/1996 5/1997	Natural light conditions	Chini Zittelli et al. (1999)
Thee-tube near horizontal tubular	36.6	0.56 ± 0.30	6/1997		
Eight-tube near horizontal tubular	97.9	0.56 ± 0.26	7/1997		
Vertical flat-plate reactors (light-paths from 1.3 to 17.0 cm)	N/A[a]	0.48[b] 1.70[c]	Summer	Natural light conditions	Zou and Richmond (1999)
Modular flat panel photobioreactor (six alveolar panels)	123	0.61–0.97[d]		Artificial illumination	Chini Zittelli et al. (2000)
Vertical flat-plate glass	200	0.24[e] 0.35–0.80[f]	Winter–summer	Natural light conditions	Richmond and Cheng-Wu (2001)
Flat-plate glass (10 cm light-path), vertical reactor	440	0.14–0.21 0.18–0.27	Winter Summer	Natural light conditions	Cheng-Wu et al. (2001)
	120 140	0.17–0.19 0.20–0.25		Artificial light conditions Artificial light conditions	
Annular reactors	140	0.25–0.35	12/99–5/00	Combined natural and artificial light conditions	Chini Zittelli et al. (2003)
	1200	0.23	1–5/99; 10/99–4/00	Combined natural and artificial light conditions	
Pyrex	0.12	0.38[g]		Artificial light conditions	Fábregas et al. (2004)
Concentric draft-tube airlift	3	1.10–1.20		Artificial light conditions	Xu et al. (2004)
GWP photobioreactors	110	0.36	Summer 2006	Natural light conditions	Rodolfi et al. (2009)
Present up-scaled experimental helical-tubular	588	1.10[h]-2.02[i] 1.21–1.63[j] 2.02–3.03[k]	6–8/07	Combined natural and artificial light conditions	ALFA project (2007)

[a] Total irradiated surfaces of all reactors: 0.52 m^2.
[b] Optimal volumetric productivity (dry weight $g\,l^{-1}\,day^{-1}$) for maximum areal productivity (dry weight $g\,m^{-2}\,day^{-1}$); optimum light-path for culturing *Nannochloropsis* in vertical reactors ca 10 cm.
[c] Maximum volumetric productivity (dry weight $g\,l^{-1}\,day^{-1}$) for minimum areal productivity (dry weight $g\,m^{-2}\,day^{-1}$); light-path in vertical reactors ca 2 cm.
[d] Mean volumetric productivity of 0.61 $g\,l^{-1}\,day^{-1}$, increasing to 0.97 $g\,l^{-1}\,day^{-1}$ with continuous illumination of one side and both sides of the panels, respectively.
[e] Optimal population density which results in the highest areal productivity in the 10 cm plate reactor.
[f] Productivities in a flat-plate photobioreactor with 1.3 and 5.2 cm of light-path, associated with low efficiency of radiation.
[g] Estimated: based on (Fábregas et al., 2004) data for *Nannochloropsis* sp. cell dry weight.
[h] Estimated: based on (Fábregas et al., 2004) data for *Nannochloropsis* sp. cell dry weight.
[i] Estimated: based on (FAO, 1996) data for *Nannochloropsis* sp. cell dry weight.
[j] Estimated: based on (Cheng-Wu et al., 2001) data for *Nannochloropsis* sp. cell dry weight.
[k] Estimated: based on (Zou and Richmond, 1999) data for *Nannochloropsis* sp. cell dry weigh.

conditions. Lipid productivity increased from 117 $mg\,l^{-1}\,day^{-1}$ in nutrient sufficient media (with an average biomass productivity of 0.36 $g\,l^{-1}\,day^{-1}$ and 32% lipid content) to 204 $mg\,l^{-1}\,day^{-1}$ (with an average biomass productivity of 0.30 $g\,l^{-1}\,day^{-1}$ and more than 60% final lipid content) in nitrogen deprived media. It is worthwhile, for comparison purposes to also refer to the *Nannochloropsis* sp. productivity of 0.09 $g\,l^{-1}\,day^{-1}$ achieved during summer period in outdoors raceways agitated by paddle wheels, almost half the productivity achieved by the same system for *Spirulina maxima* and *Chlorella vulgaris* (Gouveia and Oliveira, 2009).

The results shown in Table 2 suggest that the productivity in the range of 1.10–3.03 $g\,l^{-1}\,day^{-1}$, achieved by the present up-scaled experimental photobioreactor system, is one of the highest *Nannochloropsis* sp. productivities reported in the literature. The experimental up-scaled system was not optimised, at this stage, in terms of its full-scale manufacturing economics (i.e. materials, components, equipment, energy input) for a possible commercial application. This could be a follow-up task, depending on a possible commercial interest. As a result, carrying out economic comparisons between the up-scaled experimental system and other commercially available conventional systems (e.g. bio-fence systems) would not be justified at this stage.

4. Conclusion

A two-phase helical-tubular experimental photobioreactor suitable for controlled, continuous production of *Nannochloropsis* sp. was developed and tested under various experimental conditions. A novel automated flow-through sensor was designed which provides continuous cell concentration monitoring driving a fully automated harvesting system. Comparative results of pilot-scale trials operated outdoors suggest that the *Nannochloropsis* sp. population density reaches its maximum value under rather high temperatures, natural light intensity and duration, and within a wide pH range. Measured cellular density productivity data and estimated volumetric productivity range of 1.10–3.03 $g\,l^{-1}\,day^{-1}$, are among the highest *Nannochloropsis* sp. productivities reported in the literature.

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