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Performance of industrial-scale tubular photobioreactor in marine hatchery

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Abstract Microalgal culture is a key procedure in fish hatcheries, but it is far from optimized and faces several problems remaining to be solved. *Nannochloropsis oculata* is an important live feed alga used to rear larvae of marine finfish commonly in the aquaculture. The *N. oculata* was cultured in an experimental tubular photobioreactor with 148.4 m^2 total effective surface area and with artificial light. Scale-up experiment was completed in a hatchery. The cell density of *N. oculata* was held around 283×10^6 cells mL^{-1} after the 10th day. As a result of this study, biomass concentration spanned throughout the entire growth period and during the culture period of 70 days, totally dried by spray-dryer, corresponded to 21.357 kg dry weight biomass. This tubular photobioreactor was designed to grow algae throughout the year and especially in the main spawning season of different fish species. The results of the present study indicate that indoor tubular photobioreactor systems can be used for growing microalgae and provide many advantages comparing to open systems.

Keywords Photobioreactor · *Nannochloropsis oculata* · Microalgae · Indoor culture · Artificial light

Introduction

Aquaculture is a fast developing area, and microalgal culture is a key procedure in fish hatcheries, for the rearing of marine fish larvae with a "green water technique" (Van der Meeren and Mangor-Jensen 2007; Saka et al. 2008; Neori 2011). Microalgae are an indispensable feed source for all growth stages of bivalves and for larvae of some crustaceans and fish species in aquaculture as used directly in larval tanks. They are consumed by zooplankton, which are then consumed by fish. In this aquaculture food chain, important nutrients from microalgae are transferred to higher trophic levels via intermediary zooplankton (Brown et al. 1999; Vismara et al. 2003). Composition of microalgae, particularly their gross composition and fatty acid content (Thompson et al. 1992), is significantly alterable through culture conditions, depending especially on temperature and light conditions (Richmond 2004; Durmaz et al. 2008). In particular, microalgae production for aquaculture hatcheries faces problems in the development of a number of processes due to typically low productivity and poor quality of the resulting biomass, along with contamination and high production cost (Durmaz 2007; Muller-Feuga 2013).

Microalgal biotechnology is attracting more attention recently in different industrial areas. Various microalgae species have already been cultivated successfully with open systems. Yet, open culture systems may be contaminated with bacteria and other contaminants, which limit the usage of microalgae. Especially in fish hatcheries that contamination risk manifests itself. Furthermore, stability of the culture cell density, reliability, and persistence of the culture are other important characteristics. These lead the producers to prefer closed systems which allow better control conditions. Microalgae culture systems must be optimized, but several pending problems need to be solved. Photobioreactors have been designed in different types and shapes, and outdoor tubular and flat photobioreactors are the most popular choices, due to free light source (Borowitzka 1999; Tredici 2010; Satyanarayana et al.

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2011). From a commercial point of view, a photobioreactor must have as many of the following characteristics as possible: large productive area, high volumetric productivity, low cost, ease of control, and high reliability (Olaizola 2003).The main issues facing tubular systems are relatively higher energy costs, hydrodynamic stress, and installation costs (Borowitzka 1999; Ozkan et al. 2012).

Nannochloropsis species (Eustigmatophyta) dwell in waters with high-nutrient loading such as coastal waters and estuaries (Hibberd 1981) and are an important food source for aquatic species. They are also used as feed additive in commercial practice for larvae, juvenile molluscs, prawn larvae, and live food organisms such as rotifers. The latter, in turn, are fed to larvae of marine finfish and crustaceans. The nutritional value of microalgae is related to their biochemical composition and especially to its lipid and fatty acid composition (Sukenik et al. 1993; Durmaz et al. 2008). They are also a source of ω3 eicosapentaenoic acid that is an indispensable food chain component for organisms cultured in hatcheries (Lubzens et al. 1995). *Nannochloropsis* can create a "green-water effect" in rearing tanks (Rodolfi et al. 2003; Durmaz 2007) that improves growth, resistance to diseases, stress, and improves survivability of fish and shrimp (Vismara et al. 2003). The culture of *N. oculata* is already done in different closed photobioreactor systems such as transparent polyethylene bags, fiberglass cylinders, and flat panel reactors, mostly under artificial light, in hatcheries as feed for fish (Lubián et al. 2000; Lourenco et al. 2002; Koc et al. 2013).

Microalgae grow in nearly every biotope because of their ecological diversity and physiological adaptation (Pulz 2001). Nevertheless, different species need specific conditions such as light, temperature, and adapted culture medium. Culture systems should therefore be optimized. This study aims to describe a dynamic model of bioreactor conceived for a semi-continuous mass microalgal culture based on physiochemical and biological principles (i.e., growth medium, optimum pH for the marine hatchery).

Material and methods

Microalgae

Nannochloropsis oculata (Droop) Hibberd (CCAP 849/1) was obtained from The Culture Collection of Algae and Protozoa (CCAP), Scotland. The batch starter cultures were maintained axenically in F/2 medium (Guillard and Ryther 1962), in which nitrogen is 8.82×10^{-4} mL^{-1}, phosphate (P) 3.63×10^{-5} mL^{-1}, and pH is 7.5. This strain was inoculated in 1 L glass flasks at temperature 20 ± 1 °C, 24 h illumination with Sylvania Gro-lux 48″/40 W Wide Spectrum Fluorescent Tube-t12 at 100 μmol photons m^{-2} s^{-1} at the surface the flask measured with a Li-core 195 m. When the inocula reached a concentration of between 10^6 and 10^7 cells mL^{-1}, they were transferred to larger flat-bottom glass flasks (5 L), and when the volume reached to 200 L, the culture was inoculated to the tubular photobioreactor.

Experimental photobioreactor

The experiments were performed in a tubular photobioreactor located in an aquaculture hatchery facility in Turkey (Akvatek Company), as shown in Figs. 1 and 2. The tubular photobioreactor was wound on a rigid vertical structure, 14 m in length, 0.2 m width, and 2.65 m height and was divided into two parts: a tubular illumination receiver with a degasser and a cooler tank. The tubular illumination receiver was divided into four vertical tubular systems that were combined by manifolds. Each system was positioned in a fence-like structure made of transparent plexiglass tubes and consisted of 240 m total length with an internal diameter of 4.6 and 0.2 cm wall thickness. The total effective surface area of the tubular photobioreactor was 148.4 m^2. The degasser and cooler tank consisted of a double-walled polyester fiber tank that was used for mixing, degassing, and heat exchange of culture medium with a temperature of 18 °C controlled by an internal heat exchanger placed in titanium tubes, with a water flow of 100 L h^{-1} of sea water. The room temperature was kept at 20 ± 1 °C controlled by air conditioners. The microalgal culture circulated at a velocity of 0.6 m s^{-1} using a centrifugal pump located between the bubble column and the tubular system. The microalgae

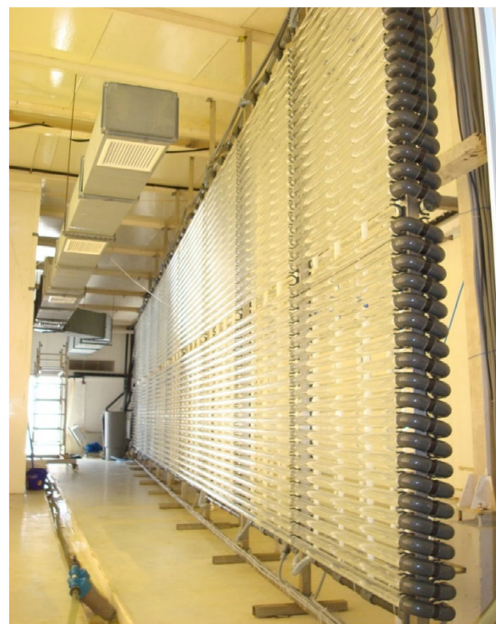

Fig. 1 View of the tubular photobioreactor at the experimental station (Akvatek Aquaculture Company, Turkey)

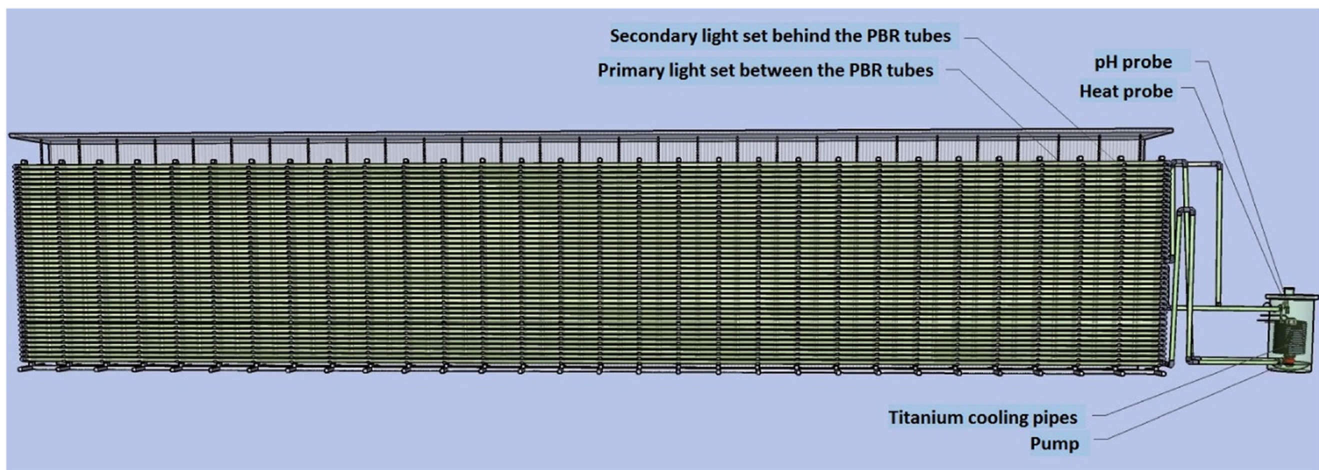

Fig. 2 Tubular photobioreactor scheme

were grown photoautotrophically with continuous mode at a dilution rate of 10–20% of total volume day^{-1}. Dilution rate was determined by specific growth rate of the culture daily. The pH and temperature were measured with a Seko PR40 pH/redox and conductivity meter (Italy) at two locations: the outlet of the tube and the degasser tank. The pH control unit was set at pH 7.5 and connected with solenoid valve for automatical injection of pure industrial-grade CO_2 gas at 5 L min^{-1}. These data were also collected by a data acquisition device.

It is not possible to completely sterilize the tubular bioreactor, but in this study, the tubular photobioreactor was disinfected by using sodium hypochlorite overnight and neutralized for 2 h with sodium thiosulfate. While preparing the tubular photobioreactor for microalgae culture, seawater was sterilized by passing through 0.02 μm filtration system, and also sterilized seawater was used for addition during the harvest period of the system.

Culture conditions

Culture was kept illuminated with 252 fluorescent lamps (Sylvania Gro-lux 48″/40 W Wide Spectrum Fluorescent Tube-t12). Two-step illumination was arranged as low light intensity (96 μmol photons m^{-2} s^{-1}) and high light intensity (200 μmol photons m^{-2} s^{-1}) according to culture cell density. Thus, possible light inhibition was avoided. The lamps were placed into glass tubes to avoid heating in the vicinity of the reactor. One group of fluorescent lamps was located between the tubes, and the other group was located behind the tubular photobioreactor. Culture medium nutrients (F/2 medium; Guillard and Ryther 1962) were added at 1 mL per liter daily. All cultures were maintained at 35 g L^{-1} salinity and temperature of 20 ± 1 °C under 24 h light regime. The tubular photobioreactor was inoculated and operated in batch mode for 11 days. Next, it was operated in semi-continuous mode by harvesting a percentage of culture volume daily which was replaced by seawater enriched with F/2 medium nutrients.

Analytical methods

Samples were collected daily for analysis of dry weight and cell counts. The dry weight of the harvested cell mass was determined using 5–10 mL of the cultures, which was filtered through predried and preweighed Whatmann GF-52 glass-fiber filters (0.45 μm, Germany) and dried overnight in an oven at 105 °C (Hu and Richmond 1994). Cell density was measured using an improved Neubauer hemocytometer, and at the same time, contamination was checked daily through visual observation. Growth rates (μ; day^{-1}) were calculated with this equation:

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t - t_0}$$

where N_t is biomass at time (t), and N_o is the beginning biomass at time t_o. The volumetric productivity, P_v (g L^{-1} day^{-1}), was calculated from the change in biomass concentration, X (g L^{-1}), within a certain cultivation period (day):

$$P_v\left(g\ L^{-1}\ d^{-1}\right) = \frac{X_2 - X_1}{t_2 - t_1}$$

The areal productivity, P_A (g m^{-2} day^{-1}), was calculated using the equation:

$$P_A\left(g\ m^{-2}\ d^{-1}\right) = \frac{V(X_2 - X_1)}{t_2 - t_1}$$

where X_1 and X_2 are the mean dry weights at time t_1 and t_2, respectively, and V is the total culture volume per 1 m^2 of illuminated culture surface area.

Harvesting and drying

Daily culture volume was taken from the culture of tubular photobioreactor system according to dilution ratio. The biomass was concentrated as 20% dry weight with a disc separator (GEA Westfalia Separator, Germany). The concentrated biomass was dried at 170 °C with a spray-dryer.

Results

The culture pH was maintained at 7.50 ± 0.2 by adding pure CO_2 regulated through a pH-stat system. As the culture progressed, increased volumes of CO_2 were required to maintain the pH at 7.50. At low cell density (less than 100×10^6 cells mL^{-1}), the CO_2 consumption was 0.4 L h^{-1} of CO_2, and when cell density rose above 239×10^6 cells mL^{-1}, CO_2 demand increased to 1.0 L h^{-1}.

At the beginning, to prevent any photoinhibition on the low cell density inoculum, light intensity was adjusted at lower level (96 μmol photons m^{-2} s^{-1}) until day 9. Mean specific growth rate was calculated as 0.30 day^{-1} for the first 8 days. Cell density increased 10 times in this period.

The variation of cell density of *N. oculata* culture under the artificial light source is shown in Fig. 3a. The cell density increased rapidly from 9.0 to 239×10^6 cells mL^{-1} on day 13 without any apparent log phase, and maximum specific growth rate was recorded as 0.70 day^{-1}. Due to high growth rate and starting the harvest regime with low rate, there was a gradual increase in the density (329×10^6 cells mL^{-1} at day 20) between day 13 and day 20. In this experiment, a semi-continuous (daily) harvesting regimen was adopted to maintain the preset cell concentration. Along with fluctuations, average cell number was held around 283×10^6 cells mL^{-1} after day 10 until the end of the experiment. After the cell number declined, harvest rate was reduced to less than 10%, allowing the culture density to reach the target amount. The daily harvest rates were altered for both persistence of the culture and protection of cells from degradation (Fig. 3b) so that the biomass concentration spanned through the entire growth period. Maximum areal productivity reached up to 3.45 g m^{-2} day^{-1}. The maximum dry biomass concentration of the harvested algae cells was 1.86 g L^{-1}, and average dry

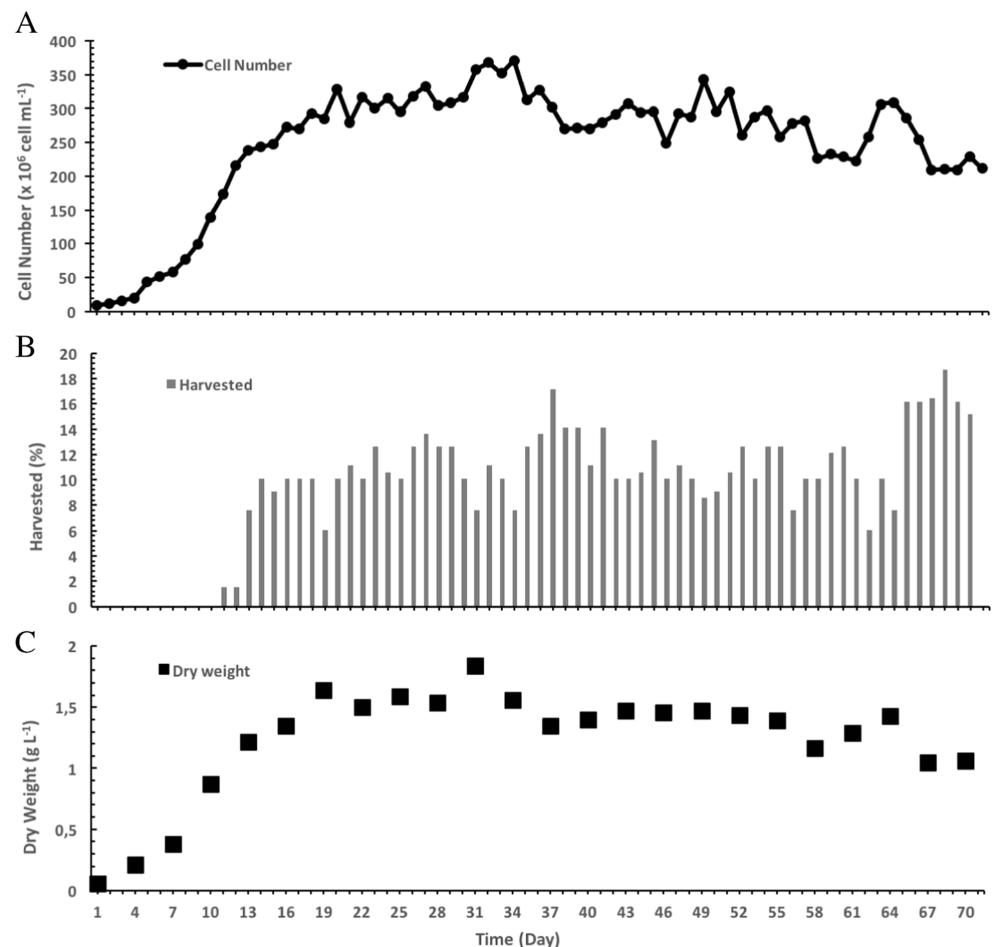

Fig. 3 The variation in cell density (**a**), harvested percentage (**b**), and dry weight (**c**) before harvest time of *N. oculata* maintained at artificial light source. *Line* indicates cell numbers ($\times 10^{-6}$ cell mL^{-1}), *bar* indicates percentage of harvested (%), and square indicates dry weight (g L^{-1})

weight during harvest time was measured as 1.43 g L^{-1} (Fig. 3c). During the culture time of 70 days, the total dry weight of *N. oculata* biomass obtained was 21.357 kg dry biomass.

Discussion

When the cell population density is too low, photodamage may cause the collapse of culture. Additionally, the culture being exposed to strong light often results in photodamage in several algal species (Hu and Richmond 1994). In this work, photoinhibition was not observed due to the application of two different light intensities according to culture cell density. Since the pH is very sensitive to changes in other variables such as total inorganic carbon and biological reactions producing oxygen, small variations occurred and were adjusted with the pH control device during the operation in semi-continuous mode.

The total biomass yield was considerably higher than algae concentrations reported for open raceway ponds, which typically range between 0.1 and 0.5 g L^{-1} (Kumar et al. 2015; Zhu 2015), but can reach up to 1.4 g L^{-1} (Ketheesan and Nirmalakhandan 2012; Ashokkumar et al. 2014). This biomass yield is comparable to typical biomass concentrations achieved in other photobioreactors. For example, in a horizontal photobioreactor, over a 165-day operation, *Nannochloropsis atomus* biomass ranged from 2.07 to 4.3 g L^{-1} (Dogaris et al. 2015).

In another studies, the volumetric productivities have been reported as 1.10–2.02 g L^{-1} day^{-1} (Coutteau 1996; Fabregas et al. 2004), 1.21–1.63 g L^{-1} day^{-1} (Wu et al. 2001), and 2.02–3.03 g L^{-1} day^{-1} (Zou and Richmond 1999) with continuous mode using combined illumination solar and artificial illumination. The average of volumetric productivity (1.44–2.23 g L^{-1} day^{-1}) shows that its estimated volumetric productivity is higher than in this study. Different illumination conditions may cause that difference. For fed-batch culture using artificial light productivities of 1.10 and 1.20 g L^{-1} day^{-1} have been reported (Xu et al. 2004).

Comparing to maximum areal productivity (3.45 g m^2 day^{-1}), it is significantly lower than the highest areal productivity in another study 23.9 g m^2 day^{-1} (Dogaris et al. 2015). This result may be due to higher surface area/culture volume ratio of our photobioreactor.

Since the light intensity in culture is related to culture depth and cell density, this relationship must be considered linear. In this study, the tubes of photobioreactors were continuously illuminated on both sides for using 4.6 cm internal diameter of tubes as it provided an advantage. The light regime prevailing in association with the narrower light-paths (less than 10 cm) could not be effective for the slow growing *Nannochloropsis* sp. cells (Zou and Richmond 2000). However, Richmond and Wu (2001) obtained the highest values of *Nannochloropsis* sp. biomass of 0.35 g L^{-1} day^{-1} dry weight with 1.3 and 5.2 cm light-paths in their study. A much higher productivity (1.43 g L^{-1} day^{-1} dry weight) was obtained in our study. In this study, an increase in biomass concentration of *N. oculata* was determined as a suitable light intensity, and light source was used for the light path length of tube. This study was completed under artificial light conditions, since direct sunlight may cause inhibition on the microalgae culture. Furthermore, artificial illumination allows for the control of illumination constantly, without any dependence on environmental conditions.

In conclusion, modern aquaculture requires year-round production capability. For this reason, fully controlled microalgae cultivation systems are necessary for the sustainability of hatcheries. Environmental conditions such as temperature and light affect microalgae cultivation. Indoor tubular photobioreactors ensure greater convenience in this regard. Hence, indoor systems are preferable with their isolation from environmental conditions.

Our results indicate that this design offers the advantage of having a large surface to volume ratio, easy control of temperature, and carbon dioxide transfer, while occupying a small ground area. In addition, fully controlled lighting ensures the persistence of production, which is not possible in outdoor systems. The growth rate and biochemical composition of *N. oculata* are subject to environmental conditions in each season. This tubular photobioreactor has been designed to provide certain microalgae species such as *N. oculata* year around in hatcheries. The authors suggest that other algal species should be tested for culture in this tubular system.

References

Ashokkumar V, Agila E, Sivakumar P, Salam Z, Rengasamy R, Ani FN (2014) Optimization and characterization of biodiesel production from microalgae *Botryococcus* grown at semi-continuous system. Energ Convers Manag 88:936–946

Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70:313–321

Brown MR, Mular M, Miller I, Farmer C, Trenerry C (1999) The vitamin content of microalgae used in aquaculture. J Appl Phycol 11:247–263

Coutteau P (1996) Micro-algae. In: Lavens P, Sorgeloos P (eds) Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper No:361, Rome, pp 7–48

Dogaris I, Welch M, Meiser A, Walmsley L, Philippidis G (2015) A novel horizontal photobioreactor for high-density cultivation of microalgae. Bioresour Technol 198:316–324

Durmaz Y (2007) Vitamin E (α-tocopherol) production by the marine microalgae *Nannochloropsis oculata* (Eustigmatophyceae) in nitrogen limitation. Aquaculture 272:717–722

Durmaz Y, Donato M, Monteiro M, Gouveia L, Nunes ML, Gama Pereira T, Gokpinar S, Bandarra NM (2008) Effect of temperature on growth and biochemical composition (sterols, α-tocopherol,

carotenoids, fatty acid profiles) of the microalga, *Isochrysis galbana*. Bamidgeh 60:188–195

Fabregas J, Maseda A, Dominguez A, Otero A (2004) The cell composition of *Nannochloropsis sp.* changes under different irradiances in semicontinuous culture. World J Microbiol Biotechnol 20:31–35

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Hibberd DJ (1981) Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). Bot J Linn Soc 82:93–119

Hu Q, Richmond A (1994) Optimizing the population density in *Isochrysis galbana* grown outdoors in a glass column photobioreactor. J Appl Phycol 6:391–396

Ketheesan B, Nirmalakhandan N (2012) Feasibility of microalgal cultivation in a pilot-scale airlift-driven raceway reactor. Bioresour Technol 108:196–202

Koc C, Anderson GA, Kommareddy A (2013) Use of red and blue LEDs and fluorescent lamps as light sources to grow microalgae in a photobioreactor (PBR). Bamidgeh 65:797–805

Kumar KS, Dahms HU, Won EJ, Lee JS, Shin KH (2015) Microalgae—a promising tool for heavy metal remediation. Ecotoxicol Environ Safe 113:329–352

Lourenco S, Barbarino E, Mancini-Filho J, Schinke K, Aidar E (2002) Effect of different nitrogen sources on the growth and biochemical profile of 10 marine microalgae in batch culture: an evaluation for aquaculture. Phycology 12:249–255

Lubián LM, Montero O, Moreno-Garrido I, Huertas IE, Sobrino C, González-del Valle M, Parés G (2000) *Nannochloropsis* (Eustigmatophyceae) as source of commercially valuable pigments. J Appl Phycol 12:249–255

Lubzens E, Gibson O, Zmora O, Sukenik A (1995) Potential advantages of frozen algae (*Nannochloropsis sp.)* for rotifer (*Brachionus plicatilis*) culture. Aquaculture 133:295–309

Muller-Feuga A (2013) Microalgae for aquaculture: the current global situation and future trends. In: Richmond A, Hu Q. Eds., Handbook of microalgal culture: Applied phycology and biotechnology. Wiley Blackwell, Chichester, pp 613–627

Neori A (2011) "Green water" microalgae: the leading sector in world aquaculture. J Appl Phycol 23:143–149

Olaizola M (2003) Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomol Eng 20:459–466

Ozkan A, Kinney K, Katz L, Berberoglu H (2012) Reduction of water and energy requirement of algae cultivation using an algae biofilm photobioreactor. Bioresour Technol 114:542–548

Pulz O (2001) Photobioreactors: production systems for phototrophic microorganisms. Appl Microbiol Biotechnol 57:287–293

Richmond A (2004) Principles for attaining maximal microalgal productivity in photobioreactors: an overview. Hydrobiologia 512:33–37

Richmond A, Wu Z (2001) Optimization of a flat plate glass reactor for mass production of *Nannochloropsis* sp. outdoors. J Biotech 85: 259–269

Rodolfi L, Zittelli GC, Barsanti L, Rosati G, Tredici MR (2003) Growth medium recycling in *Nannochloropsis* sp. mass cultivation. Biomol Eng 20:243–248

Saka Ş, Çoban D, Kamacı HO, Süzer C, Fırat K (2008) Early development of cephalic skeleton in hatchery-reared gilthead seabream, *Sparus aurata*. Turk J Fish Aquat Sci 8(2):341–345

Satyanarayana KG, Mariano AB, Vargas JVC (2011) A review on microalgae, a versatile source for sustainable energy and materials. Int J Energy Res 35:291–311

Sukenik A, Zmora O, Carmeli Y (1993) Biochemical quality of marine unicellular algae with special emphasis on lipid composition. II Nannochloropsis sp. Aquaculture 117:313–326

Thompson PA, Guo MX, Harrison PJ (1992) Effects of variation in temperature I. On the biochemical composition of eight species of marine phytoplankton. J Phycol 28:481–488

Tredici MR (2010) Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. Adv Biochem Eng Biotechnol 1:143–162

Van der Meeren T, Mangor-Jensen PJ (2007) The effect of green water and light intensity on survival, growth and lipid composition in Atlantic cod (*Gadus morhua*) during intensive larval rearing. Aquaculture 265:206–217

Vismara R, Vestri S, Kusmic C, Barsanti L, Gualtieri P (2003) Natural vitamin E enrichment of *Artemia salina* fed freshwater and marine microalgae. J Appl Phycol 15:75–80

Wu Z, Zmora A, Kopel R, Richmond A (2001) An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). Aquaculture 195:35–39

Xu F, Hu HH, Cong W, Cai ZL, Ouyang F (2004) Growth characteristics and eicosapentaenoic acid production by *Nannochloropsis* sp. in mixotrophic conditions. Biotechnol Lett 26:51–53

Zhu L (2015) Biorefinery as a promising approach to promote microalgae industry: an innovative framework. Renew Sust Energ Rev 41: 1376–1384

Zou N, Richmond A (1999) Effect of light-path length in outdoor flat plate reactors on output rate of cell mass and of EPA in *Nannochloropsis* sp. J Biotechnol 70:351–356

Zou N, Richmond A (2000) Light-path length and population density in photoacclimation of *Nannochloropsis* sp. (Eustigmatophyceae). J Appl Phycol 12:349–365