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Cell productivity of *Nannochloropsis gaditana* CCAP 849/5 in varied reactor types with different culture light paths and light regimes

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Keywords: *Nannochloropsis gaditana*, Continuous cell productivity, light-path, culture thickness

Abstract

Three biocultivators with *Nannochloropsis gaditana* (30 mm, 450 mm and 1000 mm culture thickness) were compared on continuous mode. Surface / Volume ratio was 55.2 for 30 mm, 8.7 for the 450 mm and 0.952 for 1000 mm. Cell growth showed a mean Instantaneous Growth Rate (IGR) of 0.41 and mean Cell velocity doublings of 0.59 per day for 30 mm; 0.22 IGR and cell doublings 0.32 for 450 mm; 0.15 IGR and 0.21 cell doublings for 1000 mm. Mean volumetric productivity was 1088.44 g m^{-3} d^{-1} for 30 mm, 16.71 g m^{-3} d^{-1} for 450 mm and 10.23 g m^{-3} d^{-1} for 1000 mm. Mean areal productivity was 10.10 g m^{-2} d^{-1} for 30 mm , 1.76 g m^{-2} d^{-1} for 450 mm and 5.33g m^{-2} d^{-1} for 1000 mm. Dry weight per cell was 6 pg for the 30 mm, 3.75 pg for the 450 mm and 3.5 pg for the 1000 mm. Mean plateau cell densities was 797.57 million cells per ml for 30 mm, 34.40 million cells per ml for the 450 mm and 14.62 million cells per ml for 1000 mm.

Introduction

In algae cultivation, the cost, scale and adoption rest upon the objectives of the grower and the bulk-end user. Algae needs light. Each algae cell is a tiny bio factory. How to add more light to thousands of litres in a cheap manner with lesser foot print? Science has given to the world innumerable photo bioreactors with varying geometric shapes and unit sizes. Biomass growth in a Photobioreactor (PBR) is a complex process, which is the result of multiple effects such as photosynthetic light capture, light attenuation of the suspension and reactor hydro-dynamics. In case of non-limiting nutrient conditions, light is the most relevant factor for autotrophic growth (Sforza *et al*., 2014). Light attenuation could strongly influence the photosynthetic biomass productivity. Recently, several papers are focusing on the effect of light on growth and on the modelling of light distribution in PBRs (Janssen *et al*., 2003; Gutierrez-Wing *et al*., 2012; Quinn *et al*., 2012; Gebremariam and Zarmi, 2012). In continuous cultures, resources are potentially infinite: cultures are maintained at a chosen point on the growth curve by the regulated addition of fresh culture medium. In practise, a volume of fresh culture medium is added automatically at a rate proportional to the growth rate of the alga, while an equal volume of culture is removed. The research study objective was that, three reactors are compared for *Nannochloropsis* cell productivity on continuous mode in this study viz., 30 mm thickness tubular photobioreactor indoor; 450 mm thickness tubular photobioreactor indoor; 1000 mm thick open tank reactors outdoors.

Materials and Methods

Three geometrically different modules were with different bioengineering metrics. The 30 mm culture thick tubular photobioreactor (methacrylate) is a concentric tube (30 mm culture interspacing) with a hollow inner core cylinder where internal illumination of 25,000 lux prevails at 23 ^{o} C controlled room temperature. The 450 mm thick culture is a tubular photobioreactor (92% transparent fibre plastic) having external fluorescent illumination (3000 lux) at a controlled room conditioned temperature of 23 ^{o} C and 1000 mm thick culture is an open concrete tank outdoor with 23 ^{o} C natural air temperature (with 15,000 lux by 7 AM ; 75,000 lux at midday and 15,000 lux by 7 PM). Pneumatic vertical injection rise-up of whole air cells (without causing too much growth inhibitory shear forces) allowed mixing flow in the system which helps prevent cell precipitation and enhance light utilization efficiency. Continuous culture mode of operation was performed on all the three reactor types with three replicates for each reactor type. Cultivation residence time between two harvests was 3 days with 30 mm, 5 days for 450 mm and 5 days for 1000 mm. In continuous cultures, resources are potentially infinite: cultures are maintained at a chosen point on the growth curve by the regulated addition of fresh culture medium. In practice, a

volume of fresh culture medium is added automatically at a rate proportional to the growth rate of the alga, while an equal volume of culture is removed.

Cell counts were performed daily, by triplicate, with an Improved Neubauer chamber (0.1 mm depth) to determine the cell density, specific growth rate (μ) day^{-1} (IGR) and Cell Velocity Doublings per day. Volumetric productivity and Areal productivity were calculated according to Kitto *et al*., 1999.

IGR (r) = ln Nt – ln No / t where Nt is the final cell count and No is the initial cell count; t is the number of days.

Cell doublings per day (K) = ln Nn-ln Ni / ln 2 (tn-ti) where Nn is the final cell count and Ni is the initial cell count; tn is the final time in days and ti is the initial time in days.

Volumetric productivity:

Pv (gm^{-3} d^{-1}) = (X2-X1) / (t2-t1) where X2 means the mean final dry weight of cells and X1 means the mean initial dry weight of cells.

Areal Surface productivity:

Pa (gm^{-2} d^{-1}) = V (X2-X1) / (t2-t1) where V is the culture volume per 1 m^3 of illuminated surface area ; X2 means the mean final dry weight of cells and X1 means the mean initial dry weight of cells.

Dry weight estimation:

500 ml of *Nannochloropsis* culture will be oven (laboratory-scale) dried at 80 C arranged in trays of 50 mm culture thickness. The drying would be upto 0 % residual moisture content and then weighed in an electronic balance.

Moisture content of the samples was calculated based on their weight loss at various intervals following the formula (Chakraverty, 1988): MC = (Wm / Wm + Wd) x 100

where 'MC' is the percent moisture content of the sample in wet basis (wb), Wm is the weight of the moisture present and Wd is the weight of the dried materials.

Results

The aim of the present study was also to test the continuous performance of a small-scale, continuous algae culture system in three reactor types with differential culture thicknesses – 30 mm reactor with internal illumination, 450 mm reactor with external fluoresecent illumination and 1000 mm tank reactor with external solar illumination respectively. Steady-state operating conditions were attempted and the effect of partial expulsive recycling on biomass concentration and productivity was investigated. Light energy was ensured at a constant rate, and the net flux of photonic energy absorbed by the culture was measured. The reactor performances were evaluated in terms of photosynthetic efficiencies. The light attenuation along the culture thickness was modelled to define the PAR-photosynthetically active radiation available within the culture. In 30 mm thick algae reactor, the PAR at harvest point was 600 lux. With 450 mm thick algae reactor, the PAR at harvest point was 60 lux and with 1000 mm thick culture reactor, the PAR at harvest point was 30 lux.

Performance in cell numbers for *Nannochloropsis* in 30 mm thin layer reactor showed a levelling off around 850 million cells per ml on a reactor detention of 3 days (Table 1).

Table 1: Continuous mode dry cell productivity of *Nannochloropsis gaditana* – 30mm fitoplan reactor

Cell Density on harvest	Harvest (%)	Harvest volume (litres)	Cell density post dilution (cells ml^{-1})	Cell dry wt. (pg) $cell^{-1}$	IGR (*r*)	Cell Velocity Doublings (k)	Mean Vol. Prod. $g/m^3/day$	Mean Areal Prod $g/m^2/day$
$679.0x10^6$ ±27.2	65±0	60±0	$210.7x10^6$ ±8.0	6±0				
$730.3x10^6$ ±51.5	65±0	60±0	$228.3x10^6$ ±5.8	6±0	0.41±0.02	0.60±0.02	972±68	17.6±1.2
$797.0x10^6$ ±33.4	65±0	60±0	$241.0x10^6$ ±12.2	6±0	0.42±0.01	0.60±0.01	1062±45	19.2±0.8
$817.0x10^6$ ±2.6	65±0	60±0	$246.0x10^6$ ±3.6	6±0	0.41±0.01	0.59±0.02	1089±3	19.7±0.01
$852.7x10^6$ ±17.5	65±0	60±0	$245.3x10^6$ ±8.1	6±0	0.41±0.01	0.60±0.02	1136±23	20.6±0.4
$851.7x10^6$ ±15.2	65±0	60±0	$255.0x10^6$ ±20.0	6±0	0.41±0.01	0.60±0.01	1135±20	20.5±0.4
$855.3x10^6$ ±5.5	65±0	60±0	$259.3x10^6$ ±17.2	6±0	0.41±0.03	0.58±0.04	1140±8	20.6±0.1

65% of culture volume harvested every 3 days

The 450 mm 92 % transparent acrylic cylinders staggered the cell density on harvest day between 30 and 35 million cells per ml on every five day harvest frequency cycle (Table 2).

Table 2: Continuous mode dry cell productivity of *Nannochloropsis gaditana* – 450 mm vertical tubular reactor

Cell Density on harvest	Harvest (%)	Harvest volume (litres)	Cell density post dilution (cells ml^{-1})	Cell dry wt. (pg) $cell^{-1}$	IGR (*r*)	Cell Velocity Doublings (k)	Mean Vol. Prod. $g/m^3/day$	Mean Areal Prod $g/m^2/day$
$34.6x10^6$±1.0	65±0	130±0	$10.7x10^6$±0.3	3.75±0				
33.5±0.6	65±0	130±0	$10.6x10^6$±0.4	3.75±0	0.23±0	0.33±0	16.3±0.3	1.8±0.03
$33.9x10^6$±1.0	65±0	130±0	$11.0x10^6$±0.5	3.75±0	0.23±0.01	0.34±0.01	16.5±0.5	1.8±0.05
$34.6x10^6$±0.6	65±0	130±0	$11.5x10^6$±0.2	3.75±0	0.23±0.01	0.33±0.01	16.9±0.3	1.8±0.03
$34.5x10^6$±0.6	65±0	130±0	$11.1x10^6$±0.2	3.75±0	0.22±0	0.31±0.01	16.8±0.3	1.8±0.03
$35.1x10^6$±0.6	65±0	130±0	$11.7x10^6$±0.8	3.75±0	0.23±0	0.34±0.01	17.1±0.3	1.9±0.03
$34.6x10^6$±0.6	65±0	130±0	$11.5x10^6$±0.6	3.75±0	0.22±0.02	0.32±0.01	16.8±0.3	1.8±0.03

65% of culture volume harvested every 5 days

The outdoor reactor plateaued around 14 million cells per ml on a harvest frequency of every five days (Table 3).

Table 3: Continuous mode dry cell productivity of *Nannochloropsis gaditana* – 1000 mm open tank reactor

Cell Density on harvest	Harvest (%)	Harvest volume (litres)	Cell density post dilution (cells ml^{-1})	Cell dry wt. (pg) $cell^{-1}$	IGR (*r*)	Cell Velocity Doublings (k)	Mean Vol. Prod. $g/m^3/day$	Mean Areal Prod $g/m^2/day$
$14.4x10^6$±0.8	50±0	25000±0	$6.7x10^6$ ±0.4	3.5±0				
$14.0x10^6$±1.3	50±0	25000±0	$6.5x10^6$ ±0.4	3.5±0	0.15±0.01	0.21±0.01	9.8±0.9	5.1±0.5
$14.6x10^6$±0.2	50±0	25000±0	$7.0x10^6$ ±0.2	3.5±0	0.16±0.02	0.23±0.02	10.2±0.1	5.4±0.1
$14.7x10^6$±0.6	50±0	25000±0	$7.0x10^6$ ±0.3	3.5±0	0.15±0.01	0.21±0.01	10.3±0.4	5.4±0.2
$14.9x10^6$±0.1	50±0	25000±0	$7.2x10^6$ ±0.2	3.5±0	0.15±0.01	0.22±0.01	10.4±0.1	5.5±0.0
$15.0x10^6$±0.9	50±0	25000±0	$7.0x10^6$ ±0.6	3.5±0	0.15±0.01	0.21±0.01	10.4±0.6	5.5±0.3
$14.8x10^6$±0.5	50±0	25000±0	$7.3x10^6$ ±0.2	3.5±0	0.15±0.01	0.22±0.02	10.4±0.3	5.4±0.2

50% of culture volume harvested every 5 days

IGR and Cell doublings

Fig. 1 manifests the stark elevation in IGR and Cell doublings in a thin layer reactor on continuous mode comparing thick culture reactors. Table 4 clearly depicts very high values of cell kinetics on a tabulated comparison of all the three reactors.

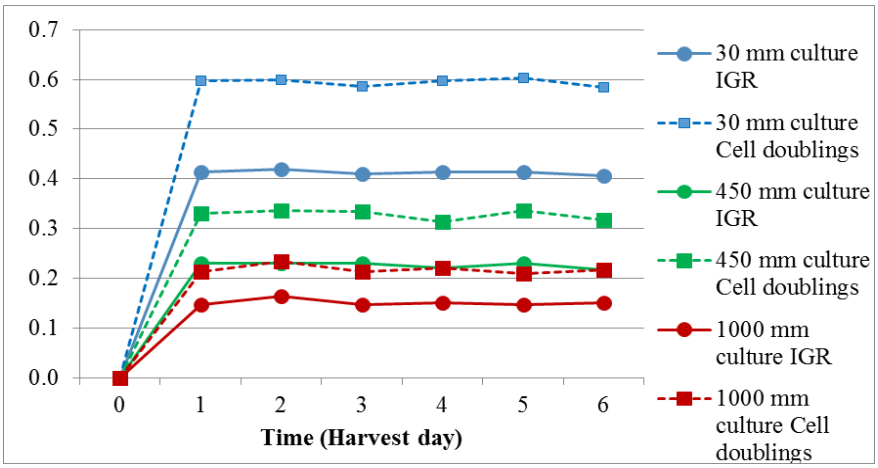


Fig. 1. Continuous mode kinetics of *Nannochloropsis* as seen in three different reactor types

Table 4. Summary of IGR and Cell doublings compared with three reactor types on continuous mode

Time (Harvest day)	30 mm culture		450 mm culture		1000 mm culture	
	IGR	Cell Doublings	IGR	Cell Doublings	IGR	Cell Doublings
0	0.00	0.00	0.00	0.00	0.00	0.00
1	0.41±0.02	0.60±0.02	0.23±0.00	0.33±0.00	0.15±0.01	0.21±0.01
2	0.42±0.01	0.60±0.01	0.23±0.01	0.34±0.02	0.16±0.02	0.23±0.02
3	0.41±0.01	0.59±0.02	0.23±0.01	0.33±0.01	0.15±0.01	0.21±0.01
4	0.41±0.01	0.60±0.01	0.22±0.00	0.31±0.01	0.15±0.01	0.22±0.01
5	0.41±0.01	0.60±0.01	0.23±0.00	0.34±0.01	0.15±0.01	0.21±0.01
6	0.41±0.03	0.58±0.04	0.22±0.02	0.32±0.02	0.15±0.01	0.22±0.02

Volumetric productivity

Volumetric productivity remains identical for every singular module due to the constancy factor of illuminated specific surface (ratio of the illuminated surface to the culture volume) (Fig.2; Table 5).

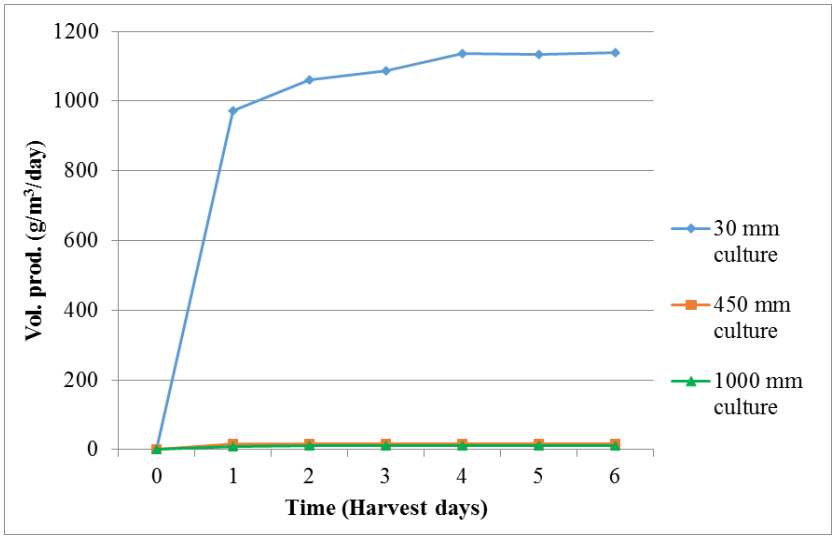


Fig. 2. Volumetric productivity of three different reactor systems studied

Table 5. Relative Comparison of Volumetric productivity on tabulation

Time (Harvest days)	Volumetric productivity (g/m^3/day)		
	30 mm culture	450 mm culture	1000 mm culture
0.00	0.00	0.00	0.00
1.00	972.33±69.06	16.30±0.26	9.73±0.90
2.00	1061.33±44.7	16.50±0.50	10.20±0.10
3.00	1088.00±3.61	16.83±0.29	10.27±0.42
4.00	1135.67±23.03	16.77±0.32	10.40±0.10
5.00	1134.33±19.76	17.07±0.28	10.43±0.57
6.00	1139.00±7.55	16.80±0.26	10.37±0.35

Areal Productivity

Fig. 3 warrants a higher productivity for 30 mm thick culture than the others. Table 6 weighs the elevated productivity for 30 mm culture against the thick 450 mm and 1000 mm cultures.

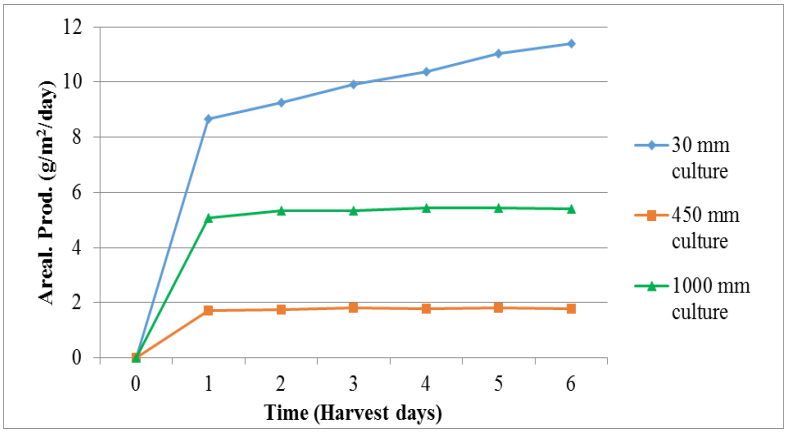


Fig. 3. Areal productivity of three different reactor systems studied

Table 6. Areal productivity comparison of three reactor types

Time (Harvest days)	Areal productivity (g/m^2/day)		
	30 mm culture	450 mm culture	1000 mm culture
0	0.00	0.00	0.00
1	8.65±1.25	1.70±0.00	5.07±0.49
2	9.25±0.78	1.73±0.06	5.33±0.06
3	9.92±0.05	1.80±0.00	5.33±0.21
4	10.38±0.40	1.77±0.06	5.43±0.06
5	11.03±0.36	1.80±0.00	5.43±0.31
6	11.40±0.15	1.77±0.06	5.40±0.20

Cell dry weight factor

Dry weight per cell as pictured in Fig. 4 exhibits a clear differential comparison of data for *Nannochloropsis* cells grown in each reactor type. The harvest cell density values are unique and characteristic for each reactor and kept levelling off under continuous culture operation (Fig. 5).

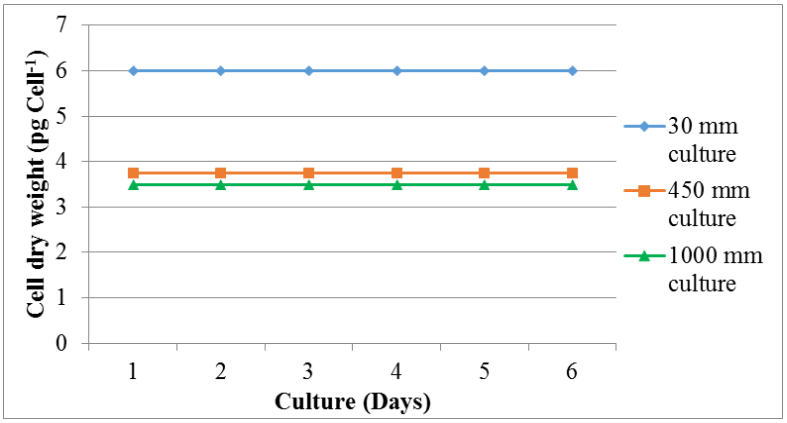


Fig. 4. Dry weight per cell levelling off at three different values for three different reactors

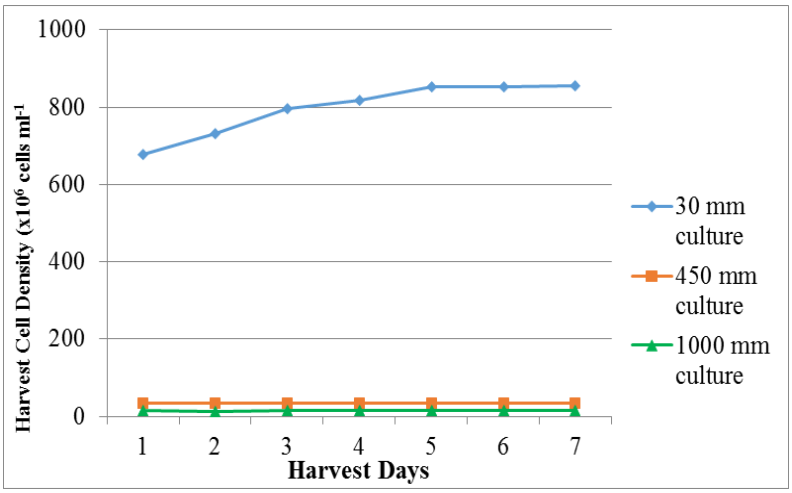


Fig. 5. Harvest Cell density curves for the three different reactors

Table 7 points out a near 20-factor fold augmentation of cell numbers in the thin layer reactor than conventional systems.

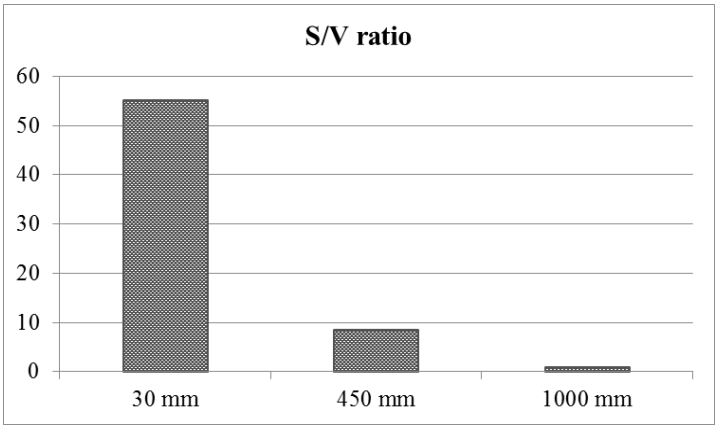
Table 7. Harvest Cell density values tabulated as a summation for all three different reactors

Harvest days	Harvest Cell Density (x 10^6 cells ml^{-1})		
	30 mm culture	450 mm culture	1000 mm culture
1	679.00±27.22	34.60±1.00	14.40±0.79
2	730.33±51.54	33.53±0.55	13.97±1.27
3	797.00±33.42	33.90±1.00	14.63±0.15
4	817.00±2.65	34.63±0.55	14.70±0.62
5	852.67±17.50	34.53±0.64	14.90±0.10
6	851.67±15.28	35.13±0.58	14.97±0.87
7	855.33±5.51	34.53±0.57	14.83±0.50

Surface / Volume Ratio

The S/V ratio of the three reactors studied gives a lucid illustration of the surface area exposition indices (Fig. 6).

Fig. 6. S/V ratio of three different reactors studied



Discussion

Light factor available per cell per unit time

Light has multiple effects on photosynthetic organisms, it provides the energy to support metabolism but it is also a fundamental signal influencing many cellular processes. Continuous culture is an attractive alternative to batch production (Bougaran *et al*., 2003) as it allows full automation of the microalgae production process, thereby reducing labor costs. Also, it provides a stable quantity and quality of microalgal biomass due to a better control of the growing environment (Borowitzka, 1997). However, appropriate equipment is required (Loubière *et al*., 2009) as well as specific knowledge of the microalgae species and their optimal culture conditions.

As light constitutes a significant proportion of the production cost (Benson *et al*., 2009), its supply should be adapted depending on the photobioreactor geometry and dilution rate (Marchetti *et al*., 2012). Cell weight of *Nannochloropsis* may represent an important parameter in production of food chain components for aquaculture. The light path, conceivably through its effect on the light regime, exerts a significant effect on cell weight of *Nannochloropsis*. The length of the light-path exerted a strong effect on the optimal cell density of *Nannochloropsis* (Zou and Richmond, 1999).

In order to investigate light influence on *Nannochloropsis* growth, culture reactors with varying light paths was selected. The aim of the work was not to achieve the maximal productivity but to continue the algae in the exponential phase. The duplication rate of *N. gaditana* is not light limited but depends on other factors like CO_2 and nutrient availability. The microalga *N. gaditana* has a remarkable capacity of acclimating to different photon fluxes by adapting its photosynthetic apparatus. In the outdoor reactor environment, incident light intensity is highly variable. Thus, in the perspective of exploiting this alga for a large scale cultivation, in order to avoid the limitation in productivity imposed by photo inhibition, cells should be maintained in a state of active duplication. Cells adapted to high light can exploit a higher fraction of incident light for photochemistry with respect to cells acclimated to low light. *N. gaditana* cells acclimate to different light regimes by accumulating different amount of pigments (Simionato *et al*., 2011).

N. oculata contain the pigments chlorophyll a, b-carotene, and the xanthophylls, violaxanthin and vaucherxanthin but lack chlorophyll b (Cohen, 1999). The pigment concentrations of *Nannochloropsis* sp. depend also on the PBR thickness and the initial cell concentration (Fisher *et al*., 1996; Zou and Richmond, 2000). Zou and Richmond (2000) showed that *Nannochloropsis* sp. had an order of magnitude larger steady-state chl a concentration per cell in cultures grown in 3cm thick PBRs compared with those grown in 1cm thick PBRs both exposed to 3000 lmol/m^2s. However, cells grown in1cm thick PBR had a larger carotenoid to chl a ratio. Pegallapati and Nirmalakhandan, 2013 devised an Internally illuminated photobioreactor for algal cultivation under carbon dioxide-supplementation. But Ian Laing (1990) from CEFAS, UK started the first internally illuminated reactor.

Areal Productivity

Light chamber geometry is a dominant criterion for optical photosynthetic performance of algal cells During acclimation at high light intensities, photosynthetic organisms adopt two strategies for modulate light harvesting efficiency and their pigment content: the first one is the increase of the antenna size, *i.e.* the number

of chlorophyll/proteins of the light harvesting system associated to each reaction center; the second is increasing the number of reaction centers per cell (Falkowski and Owens, 1980; Walters, 2005). Marine microalgae tend to extinguish and defuse the hypersaturating photon fluence rate by maximizing cell biomass, promoting auto-shading of cells to achieve lumostatic conditions at steady state.

Cell dry weight factor

The net dry cell mass output is a mere function of the Instantaneous growth rate and algal cell concentration in culture. The length of the reactors light path always exerts a decisive effect on cell response to light through its influence on the light regime in culture. High cell density commencement of cultures causes a very low photosynthetically active radiation inside the culture and induces formation of light pigments in order to harvest more light for photosynthesis. Low cell density initiation of cultures increases the photon flux density (illuminated cells per unit volume) resulting in abnormal increase in cell density in numbers.

Surface / Volume Ratio

In outdoor micro algal culture, all incident light energy is absorbed in the photic zone, the upper 1-3 cms of the cell suspension. Deeper cultures would not result in higher productivity (Ben-Amotz and Avron, 1989). The availability of light in many instances limits the productivity that can be attained in an outdoor pond (Ben-Amotz and Avron, 1989). Productivity depends not only on the total irradiance impinging on the culture surface, but also and more importantly, on the amount of energy available at the cell level (Richmond, 1996).

For a given set of conditions, there is an optimum value of population density which yields the highest output rate (Chini Zittelli *et al*., 1996; Richmond, 1992). Cell density is especially relevant, since its adequate manipulation represents the best way of modifying the amount of light available for each cell in the culture (Vonshak *et al*., 1982).

Conclusion

The light path, conceivably through its effect on the light regime, exerts a significant effect on cell weight of *Nannochloropsis*. The length of the light-path exerted a strong effect on the optimal cell density of *Nannochloropsis.* Productivity was dependent not only on the total irradiance impinging on the culture surface, but also on the amount of energy available at the cell level. The S/V ratios gives a lucid illustration of the surface area exposition indices in varied photobioreactors.

Benefits of this research

1. Capacity building on dry weight awareness of *Nannochloropsis*.
2. The dry matter values would help for correlating the future aquaculture nutrition studies for applied biofloc practices where *Nannochloropsis* is employed for water conditioning effects.
3. Advancing knowledge on the significance of culture light path for future algae cultivation program indoors in fish hatcheries.
4. Rating the quality and quantity of *Nannochloropsis gaditana* for various utilities (pond bloom ; fish larval feeding ; rotifer enrichment *etc*)
5. Improvement of existing outdoor reactor practices and correction of procedures for maximised cell dry matter accrual outdoors.

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