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Effect of summer / winter light intensity and salt on growth kinetics and beta carotene accumulation by *Dunaliella* in open outdoor earthern ponds in a desert island, off UAE coast.

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Abstract

The optimization of the operation under the climatic conditions of a coastal island in United Arab Emirates on an experimental 20,000 m^2 open earthern pond plant for beta carotene production by *Dunaliella* has been pursued. The effect of culture depth, cell density and dilution cycles on total carotenoids and beta carotene biomass productivity were studied under a semi continuous culture regime in open unmixed earthern ponds outdoors. Experiments were carried out for both seasonal extremes (summers and winters). Dry beta carotene per cell was sustainable between 200 and 250 psu recording 90-95 pg / cell. Total dry carotenoid of 6.0 – 6.9 mg / litre of *Dunaliella salina* cells was scored between 200 – 250 psu values for salinity. A standing dry cell crop productivity of 0.93 g / m^3 / day was observed during summers with a harvestable dry cell crop productivity of 0.31 g / m^3 / day. The results obtained have validated this location and the operating conditions established as being most appropriate for mass production of beta carotene rich *Dunaliella salina*.

Key words: *Dunaliella*, open outdoor ponds, betacarotene accumulation

Introduction

Unicellular green algae of the genus *Dunaliella* (Chlorophyta) are ovoid in shape, lack a rigid cell wall, contain one large cup-shaped chloroplast and are motile with two long flagella (Borowitzka and Borowitzka, 1992). *Dunaliella* are probably the most halo tolerant eukaryotic organisms showing a remarkable degree of adaptation to variety of salt concentrations from as low as 0.2 % to 35 %; it is the only eukaryotic photosynthetic organism that has been detected in significant numbers in concentrated saline lakes (Ginzburg and Ginzburg, 1985; Ben Amotz and Avron, 1990; Ben Amotz, 1991). Because *Dunaliella* contains abundant beta carotene, the algae has been used as a food coloring agent, a pro Vitamin supplement for food and animal feed, an additive to food and cosmetics as well as an anti oxidant etc (Edge *et al*., 1997; Johnson and Schroeder, 1995).

Dunaliella production facilities are located in areas where solar output is maximal, cloudiness minimal; climate is warm and hypersaline water available (Ben Amotz, 1999). Abu Dhabi, United Arab Emirates meets many of these conditions. This situation would justify the establishment of *Dunaliella* production facilities at Al Futaisi Island, thus offering new biosaline opportunities in a virgin desert land. Futaisi farms are open unmixed ponds and have the lowest cost algal production in the world, use an efficient, reliable and low cost culture process (low pond construction costs & minimal energy and labour requirements for culture maintenance. Economic viability of this system seems to rest on low land costs, as well as on the fact that water is free other than for low energy pumping costs and that harsh climate is close to optimum for *Dunaliella* cells. Production can thus be maintained all year round.

In order to establish the performance of a proto type pilot plant for *Dunaliella* production in the considered area, research on controllable physical factors (culture depth, cell density and dilution cycles) that affect productivity are needed. Furthermore, the study of the influence of climatic parameters on growth and carotenoid production during the whole year cycle is also great interest to assess the suitability of the region for the purpose of mass cultivation of Beta carotene rich *Dunaliella.* These were actually the aims pursued in the present work, the results of which are underlying the interest and opportunity of mass cultivation of this alga for Beta carotene production off UAE coast.

Materials and Methods

Dunaliella salina [brought by senior author, which was originally received by Abu-Rezq from Professor Borowitzka and brought to Kuwait (Abu-Rezq *et al*., 2010)] was used in the farming trials at Al Futaisi island, Abu Dhabi, UAE. The objective of this research study was to evaluate the cell productivity in terms of cell numbers, cellular beta carotene biomass, the Instantaneous Growth Rate and cell doublings per day. Under prior optimization trials, it was found that during summers, 23 % of the standing crop harvest and dilution every four days was sustainable with a mean incident light radiation of 100,000 lux. During winters, 11 % of the standing crop harvest and dilution was sustainable with a mean incident light radiation of 65,000 lux. Carotene-turgid cells were less-motile and wind-carried to the pond corners (Plate 1). The average depth of the pond was fixed at 30 cms during summers and 20 cms during winters on an empirical approach.

Plate. 1. Carotene-rich *Dunaliella* cells suffering wind-carriage effects to pond corners.

Plate 2. Vast expanse of solar captor surface of 20,000 m^2 pond of *Dunaliella*.

Temperature and light were not controlled and reflected those available naturally. Cells were grown in semi-continuous regime with post harvest dilutions as specified above. The increase in biomass and Beta carotene between two consecutive dilutions was taken as a measurement of cell growth and biomass. The photosynthetic photon flux density (PPFD) at the culture surface was measured using a quantum sensor (model LI-190 SB); connected to a quantum / radiometer / photometer (model LI – 188 B; Li – Inc., Lincoln NE, USA). Cell density was determined from the measured cell number, during the logarithmic phase of growth.

Dry weight was determined in pre-washed glass fibber filter with aliquots of the culture, washed with ammonium formate 1% (W/V) and dried at 80 C for 24 h.

For carotene analysis, a 1 ml aliquot of suspension was centrifuged at 1000 x g for 5 min and the pellet extracted with 3 ml ethanol : hexane 2:1 (v/v), 2 ml water and 4 ml hexane were added and the mixture vigorously shaken and centrifuged again at 1000 x g for 5 minutes. The hexane layer was separated and its absorbance determined. A 450 x 25.2 equals the micrograms of carotene in sample (Shaish *et al*., 1992).

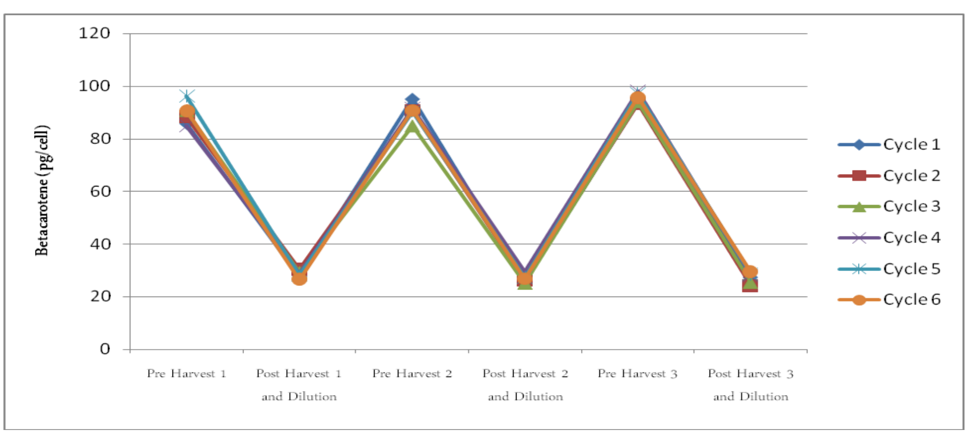
Results

Total dry carotenoids (mg/L) on semi continuous harvest - dilution cycles during summers (100,000 lux) at 250 psu showed a highest pre-harvest value of 6.93 mg / L and a highest post-harvest value of 2.7 mg / L, when harvest and dilution were 23 % of the standing crop (Table 1). Summer harvest frequency was every 4 days. The concentration of Beta carotene present (pg/ cell) on semi continuous harvest – dilution cycle during summers (100,000 lux) at 250 psu exhibited uniformity in dry beta carotene values / cell. However the maximum pre-harvest value recorded were 98.14 (pg/cell). The lowest pre harvest value was 84.88 (pg/cell). Post harvest and dilution reading recorded the maximum of 30.47 (pg/cell) and a lowest reading of 24.13 (pg/cell)(Fig. 1).

Table 1. Total dry carotenoids (mg/l) on semi-continuous harvest-dilution cycles during summers (100,000 Lux) at 250 psu

Time Period	Before Harvest	Post Harvest (23% Harvest and Dilution)	Before Harvest	Post Harvest (23% Harvest and Dilution)	Before Harvest	Post Harvest (23% Harvest and Dilution)
Cycle 1	6.93	2.54	6.86	2.47	6.14	2.56
Cycle 2	6.34	2.40	6.06	2.39	6.01	2.29
Cycle 3	6.87	2.43	6.48	2.45	6.18	2.36
Cycle 4	6.78	2.57	6.54	2.37	6.84	2.38
Cycle 5	6.79	2.65	6.45	2.73	6.81	2.49
Cycle 6	6.91	2.70	6.31	2.70	6.10	2.19

Fig.1. Concentration of betacarotene (pg/cell) on semi continuous harvest-dilution cycles during summers (100,000 Lux) at 250 psu.

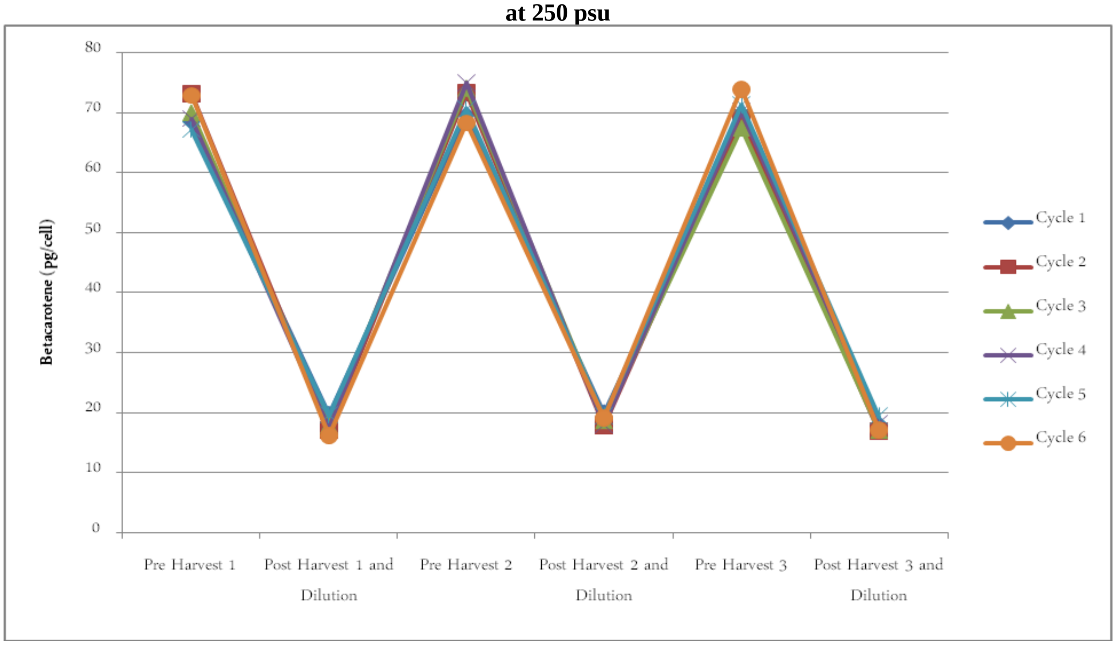


Total dry carotenoid (mg/l) on semi continuous harvest-dilution cycles during winter (65,000 lux) at 250 psu showed a highest pre-harvest value of 5.73 mg/ l and a highest post harvest value of 2.01 mg / l, where harvest-dilution were 11 % of the standing crop (Table 2). Winter harvest frequency was every 10 days. The concentration of beta carotene present (pg/cell) on a semi continuous harvest-dilution cycle during winter (65,000 lux) at 250 psu exhibited similar relative consistency in results. However, the maximum pre harvest value recorded was 75.02 pg / cell. Post harvest and dilution readings recorded a maximum of 19.99 pg / cell (Fig. 2).

Table 2. Total dry carotenoids (mg/l) on semi-continuous harvest-dilution cycles during winters (65,000 Lux) at 250 psu

Time Period	Before Harvest	Post Harvest (11% Harvest and Dilution)	Before Harvest	Post Harvest (11% Harvest and Dilution)	Before Harvest	Post Harvest (11% Harvest and Dilution)
Cycle 1	4.80	1.90	4.74	2.01	4.81	1.96
Cycle 2	4.79	1.84	4.90	1.93	5.17	1.88
Cycle 3	4.98	1.91	5.03	1.87	5.64	1.78
Cycle 4	4.99	1.79	5.33	1.83	5.18	1.69
Cycle 5	4.72	1.78	5.73	1.97	5.71	1.81
Cycle 6	4.88	1.93	1.89	1.92	1.87	1.79

Fig.2. Concentration of betacarotene (pg/cell) on continuous harvest-dilution cycles during winters (65,000 Lux) at 250 psu

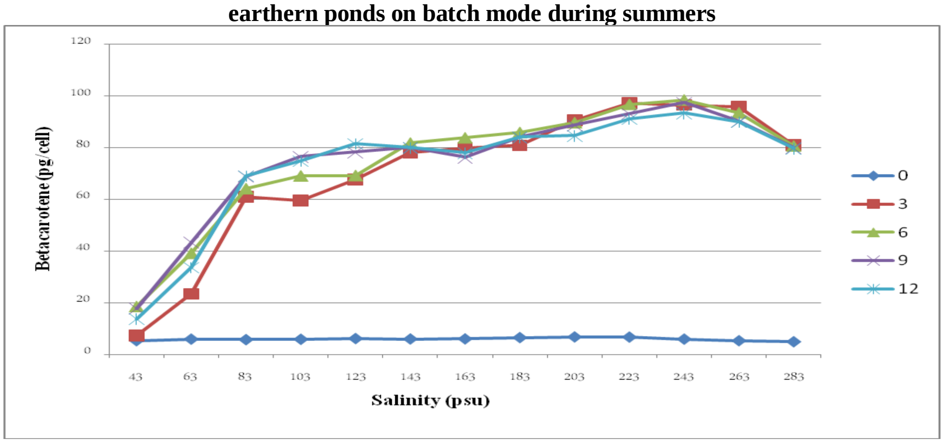


The next step studying the total dry carotenoids (mg / l) at different salinities, outdoors on batch mode during summers, it was clearly evident that 263 psu possessed the highest cellular total dry carotenoids of 6.9 mg/l (Table 3). Analyzing the concentration of beta carotene (pg/cell) of *Dunaliella salina* (AUS) at different salinities in outdoor earthern ponds on batch mode during summers, it revealed that 243 psu marked the highest concentration of 98.34 pg/cell (Fig. 3).

Table 3. Total dry carotenoids (mg/l) of Dunaliella salina (AUS) at different salinities in outdoor earthern ponds on batch mode during summers

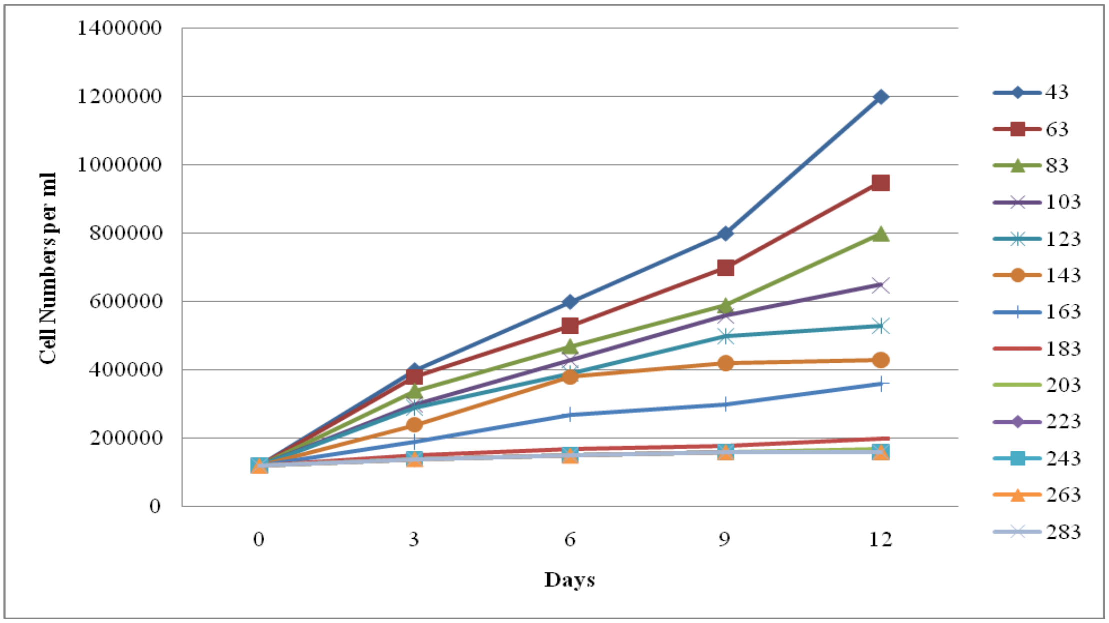
Days	Salinity												
	43	63	83	103	123	143	163	183	203	223	243	263	283
0	3.08	3.27	3.37	3.47	3.52	3.79	3.89	3.94	4.08	4.48	3.99	3.84	3.99
3	3.66	3.99	4.10	4.13	4.23	4.48	4.60	4.88	5.41	5.54	5.02	4.62	4.06
6	4.40	4.60	4.65	4.70	5.30	5.70	5.80	6.00	6.20	6.40	6.70	6.90	5.70
9	4.50	4.60	4.80	4.90	5.38	5.75	5.90	6.00	6.20	6.30	6.50	6.90	5.75
12	4.60	4.70	4.80	5.10	5.56	5.80	5.90	5.80	5.90	6.00	6.50	6.70	5.40

Fig. 3. Concentration of betacarotene (pg/cell) of *Dunaliella salina* (AUS) at different salinities in outdoor earthern ponds on batch mode during summers



Performance in cell numbers of *Dunaliella salina* (AUS) at different salinities in outdoor earthern ponds on batch mode during summers depicted cumulative increase in cell count from 120,000 cells / ml on day 0 to the highest achievable count of 1.2 million cells / ml on day 12 at 43 psu. These numbers accrued, progressively reduced with increasing salinities. However, growth in numbers apparently attenuated beyond 223 psu (Fig. 4).

Fig. 4. Performance in cell numbers of *Dunaliella salina* (AUS) at different salinities in outdoor earthern ponds on batch mode during summers.



Instantaneous Growth Rate (IGR) of *Dunaliella salina* (AUS) at different salinities in outdoor earthern ponds on batch mode during summers proved responsive only at 143 psu as shown by the equation,

$Y = - 0.026\ X + 0.3\ r^2 = 0.941$

Doublings per day (DPD) of *Dunaliella salina* (AUS) at different salinities in outdoor earthern ponds on batch mode during summers was found steady at 143 psu as shown by the equation,

$Y = - 0.037\ X + 0.435\ r^2 = 0.954$

Discussion

The coastal areas of UAE includes several small shoals, islands, protected lagoons, channels and deltas, an inner zone of intertidal flats (where algal mats are well-developed) and broad areas of supra tidal salt flat designated as 'sabkha'. In a depositional environment like the sabkha, with strong evaporation, the pore waters become highly concentrated and are drawn towards the surface, causing the precipitation of evaporates – halite, gypsum, anhydrite etc. Futaisi, one of the group of barrier islands that stretches along much of the coastline from Ras Ghurab to the Dabbiya peninsula, lies in the lee of the barrier island of Bahrani, in a shallow sheltered lagoon al complex to the southwest of Abu Dhabi islands, five kilometres away. Around 10 kilometres long and 5 kilometres wide, Futaisi island has a mosaic of different coastal habitats including wind-blown sand and shell sands, wind-scoured rocky outcrops, sabkha (salt flats), intertidal flats and mangrove along a 30 kilometre shoreline. Zeuge (pleistocene carbonates) of quaternary period aeolianite remnants with capping of marine strata and sediments are still evident.

The climatic condition for United Arab Emirates especially in the coastal island regions with up to 8400 hours of sunlight per year and a mild temperature regime with sea fanning breezes provided an excellent environment for mass production of beta carotene-rich *Dunaliella*. This is substantiated by results showing a sustained and significant cell growth and biomass during the summer season, with lag observed during winters.

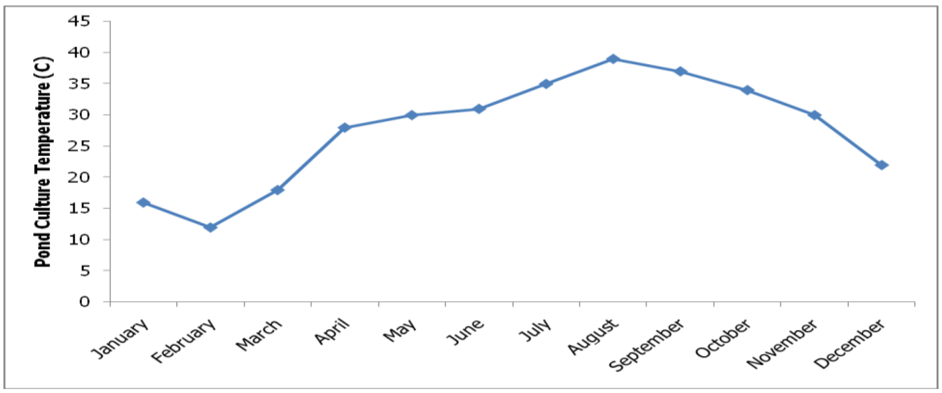
Solar radiation provides the sole energy source for the autotrophic *Dunaliella*, for which irradiance represents a major and decisive factor influencing growth, as well as induction of beta carotene accumulation (Borowitzka, 1992). The availability of light in many instances limits the productivity that can be attained in an outdoor pond (Ben-Amotz and Avron, 1989). However, the relatively uniform incident irradiance throughout the year at Futaisi Island indicates that it should be possible to maintain significant productivity all the year, allowing uninterrupted use of the production facilities. The values for biomass productivity at different seasonal extremes in the 20,000 m^2 ponds studied support this view.

The productivity of outdoor micro algal cultures is also affected by some manageable parameters (eg. turbulence, depth, cell density or dilution cycles), an adequate combination of which allows better use of the incident sunlight, with a consequent effect on productivity (Richmond, 1986; Vonshak *et al*., 1982).

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 andAvron 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). The best results in summer were obtained for 30 cms depth and for winters at 20 cms depth.

Nevertheless, in the operation of large scale ponds, engineering restrictions make it essentially impossible to maintain a uniformly smooth pond bottom to operate at small depth values; In practice, most commercial *Dunaliella* ponds have a depth of between 10 and 20 cms (Ben Amotz, 1999). In the farm under study presently at Al Futaisi Island, the 20,000 m^2 pond was operated at 20 to 30 cms depth all through the year. Temperature of culture pond showed a marked rise during summers and a notable reduction during winters (Fig. 5).

Fig. 5. Average monthly pond culture temperature throughout the year during study period.



Cell density also represents a basic parameter in determining the production of photoautotrophic mass exerting far-reaching effects on the general performance and productivity of the cultures. The Australian strain studied outdoors in UAE showed a maximum cell density of 1.2 million cells / ml on day 12 at 43 psu., but performed maximum accrual of 2.9 x 10^6 cells ml^{-1} at day 12 indoors in flask cultures in Kuwait at 45 ppt (Abu Rezq *et al*., 2008). For a given set of conditions, there is an optimum value of population density which yields the highest output rate (Chini Zittelli *et al*., 19965; 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). Cell mixing velocity in the present 20,000 m^2 ponds is subject to wind-aided crest / trough rippling effect on surface.

The results obtained by Abu Rezq et al., 2010 regarding the growth rate of *D. salina* (Australian) cultured at different light intensities (10 and 18x10^3 lux) showed that growth also increased with increasing light intensity, indicating that this alga prefers high light intensity to low light intensity producing up to 3.79x10^6 cells ml^{-1} at 18x10^3 lux and only up to 2.40x10^6 cells ml^{-1} at 10x10^3 lux. As the same Australian strain was brought to Abu Dhabi by the senior author (MRK), similar behavior was repeated at outdoors too in the present study. Australian strain performed better in flask culture growth at 50 psu than 200 psu in Kuwait (Abu-Rezq et al., 2009). The same behavior was repeated outdoors in UAE with the same Australian strain.

In our 20,000 m^2 pond facility (Plate 2), the population density at a carotenogenic salinity of above 200 psu staggered at 0.16 million cells / ml. However, at lower salinities (<200 psu), there is an obvious penalty of lower dry carotene / cell, but with good cell numbers. Supra higher salt concentrations registered higher dry carotene per cell but witnessing a serious cut in cell numbers. Higher residence time of 10 days during winters, though effected seriously higher nitrogen starvation, did not appreciably increase the dry carotene / cell. Detention time less than 10 days did not accelerate cell growth during winters. In the present study, summer harvestable dry cell crop productivity of 0.31 g / m^3 / day was observed without paddle-wheel agitation but relying on natural wind ripples. In Southern Spain, with paddle-agitated cultures, an average annual productivity of 1.65 g (dry wt) m^{-2} d^{-1} was estimated for *Dunaliella* biomass, being that for beta-carotene of about 0.1 g m^{-2} d^{-1}. The production scale attempted here in this study is comparable to that of a commercial open unmixed earthern pond facility. However, further research is wanting for the smooth line optimization of operating conditions.

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