



Effects of Environmental Factors on the Growth, Optical Density and Biomass of the Green Algae *Chlorella Vulgaris* in Outdoor Conditions

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ABSTRACT: Effect of environmental factors on the growth of the *Chlorella vulgaris* was studied. *C. vulgaris* was cultivated in sterilized natural seawater enriched with F/2-Si medium. Then grow in bucket, tub and photobioreactor (PBR) in outdoor condition. The daily routine work consisted of culture checkups of optical density, biomass gains, atmosphere lux, culture lux, atmosphere temperature and culture temperature were recorded. The highest biomass yields were ($3.0 \mu\text{g/ml}^{-1}$) in December and ($2.01 \mu\text{g/ml}^{-1}$) in November in PBR. The highest deviation was in atmosphere lux in time 8:30 (± 117.7) and lowest deviation was in atmosphere temperature in time 15:00 (± 1.0499). Optical density (OD) indicated that the best growth of *C. vulgaris* in outdoor condition was obtained in 650 lux and also it increased with increasing amount of lux. Tub report of *C. vulgaris* showed different growing behaviors at the various concentration of light and at the different temperatures. Algal production in outdoor PBR is relatively inexpensive, but is only suitable for a few, fast-growing specie. Finally, this fact is noteworthy that in outdoor conditions, temperature and light have important role in growth of *C. vulgaris* in present study. ©JASEM

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KEY WORDS: *Chlorella vulgaris*, Biomass, Photobioreactor, Growth.

Introduction

Nowadays with increasing the population, the importance of microalgae to supply food and energy is universally recognized more than ever before. However issues concerning sustainable and practical mechanism for culturing microalgae can be found almost in all over the world. Algae divided two photosynthetic organisms such as microalgae and macroalgae and they are often called primary producers because they are the important source of both cellular carbon and chemical energy for other organisms (Sirakov et al., 2015). Microalgae is one of the main producers of organic matter in aquatic ecosystems that inhabit the surface sunlight layer of fresh, brackish and salt bodies of water (Collier et al., 1978). Ecological requirements such as light, temperature or salinity are effective on the presence and growth intensity of the individual species and phytoplankton groups (Collier et al., 1978; Orus et al., 1991). Microalgae biomass consists of about 50% of carbon on a dry weight basis and also carbon dioxide is used for producing carbon in cells (Sanchez Miron et al., 2003). In the production of biomass, the importance of photosynthetic microalgae is more than plants that use light energy and carbon dioxide (Benemann, 1997; Miao and Wu, 2006). Microalgae are also crucially dependent on minerals such as macronutrients and micronutrients. They may be used

to different applications, such as purification of wastewater under either autotrophic or mixotrophic conditions (Olaizola, 2003; Munoz and Guieysse, 2006), diesel replacements (i.e., coal, oil, and gas) (Illman et al., 2000), pharmaceutical products, food for aquaculture and are necessary in aquatic food webs (Spolaore et al., 2006). Changes in growth conditions (temperature and light intensity) or nutrient media characteristics (concentration of nitrogen, phosphates, and iron) can impact on quantity and quality of cells lipids (Mikulski 1982; Liu et al. 2008). *Chlorella vulgaris* is a small green microalga. It size is 2-10 μm in diameter and has a high content of about 51-58% protein, 12-17% carbohydrate and 14-22% lipid (calculated as % of dry matter) also it growth depends on environmental factors (Spolaore et al., 2006). The study of Seyfabadi et al. (2010) showed a protein concentration in *C. vulgaris* with different conditions of light regimes can be reached of 33-46%. Putt (2008) reported under certain environmental factors the lipid content of *C. vulgaris* can be up to 30%. One of the important properties of *C. vulgaris* is that it can be grown both autotrophically (the cell collects the energy from the light through photosynthesis) and heterotrophically (the cell only requires a carbon source as energy) (Seyfabadi et al., 2010; Larsdotter, 2006). Molina et al. (2000) reported that the used of PBRs for producing large amount of microalgae

biomass are successful. There are several other algae that could be of interest in a study like this. Different algae have different condition and required a certain environment factors for growth. Open ponds are one of the primary and effective methods in large-scale and PBR is the alternate method for the production of microalgae (Lebeau and Robert, 2003). It can be installed indoors, receiving artificial or be prepared outdoors, getting visible light (Matos et al., 2014). In present experiment evaluated the effect of the main environmental factors such as light, temperature and lux on optical density, biomass and growth of *Chlorella vulgaris* in outdoor conditions in bucket, tub and PBR in Qeshm Island in Iran.

MATERIALS AND METHODS

Pure algal cultures were isolated from natural plankton communities of laboratory of biotechnology company in Qeshm Island in Iran where our outdoor area was there. Annual temperature in Qeshm Island ranged from 13°C to 39°C and is rarely below 10°C or above 40°C. The *C. vulgaris* cultures were cultivated in sterilized natural seawater enriched with F/2-Si medium which contains NaNO₃ 75 mg, NaH₂PO₄ 2H₂O 5.65mg, Na₂ EDTA 4.16 mg, FeCl₃ 6H₂O 3.15mg, CuSO₄ 5H₂O 0.01 mg, ZnSO₄ 7H₂O 0.022 mg, CoCl₂ 6H₂O 0.01 mg, MnCl₂ 4H₂O 0.18 mg, Na₂MoO₄ 2H₂O 0.006 mg, Vitamin B12 0.0005 mg, Vitamin B1 0.1 mg and Biotin 0.0005 mg per liter as described by Guillard (1962). *C. vulgaris* culture grew at first in indoor in lab after that transferred in outdoor condition in large volume in bucket, tub and PBR (cylindrical PBR, with vertical arrangement of the cylinder) Fig.1. The OD and pH should be noted every morning and evening. Daily routine work was culture checkups in terms of OD, biomass gains, atmosphere

lux, culture lux, atmosphere temperature and culture temperature. Culture pH should be adjusted with CO₂ gas whenever it is required. It should be always maintained at 7.0 and should not go beyond 7.5. In emergency condition, pH can be adjusted with 5%-10% phosphoric acid. This maintenance is applicable for all the outdoor and large scale growth of the *C. vulgaris* cultures. Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (e.g. in outdoor cultures) and to improve gas exchange between the culture medium and the air. For high dense cultures, the CO₂ derived from the air (containing 0.03% CO₂) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply (e.g. at a rate of 1% of the volume of air). CO₂ addition furthermore buffers the water against pH changes as a result of the CO₂/HCO₃⁻ balance. Depending on the scale of the culture system, mixing is achieved by daily stirring by hand (tubes, Buckets) and aerating (PBRs). In this study, growth experiments were done at different temperatures and lights in two times 8:00 AM and 15:00 PM in four months December, November, March and April in 2011. Algal daily growth changes measured in optical density at 500 nm with a spectrophotometer (UV757CRT) (Guillard, 1962). Cell numbers (cells mL⁻¹) were counted under a microscope with a haemocytometer. Biomass concentration (dry weight per liter) was separated from the medium by centrifugation at 5000 rpm for 15 min, using a centrifuge model 42426 (ALC, Milan, Italy) then dried at 105°C for 48 h, pulverized in a mortar and stored at 20°C for later analysis.

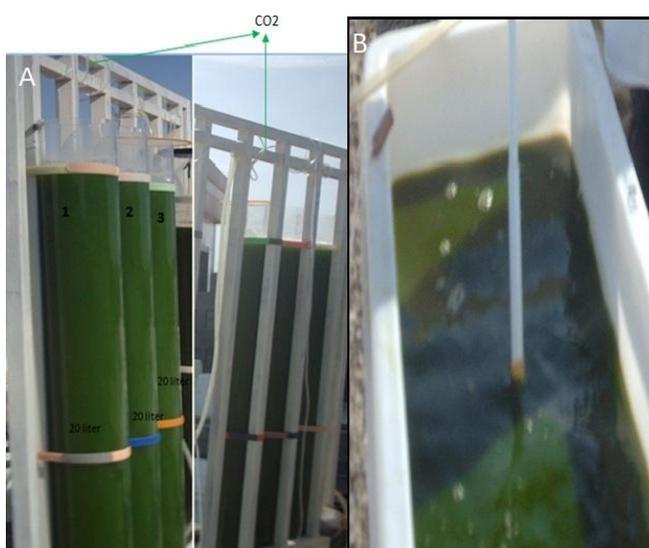


Fig.1. Growth of algae in photobioreactor (A) in tub (B)

The growth of microalgae is characterized by the following five phases (Fig. 2). Induction phase

During induction phase, a little increase observed in microalgae cell density and in this stage cultures have short log phases that can decrease the time needed for up scaling (Carlsson et al., 2007). Exponential phase
During the second phase, the cell density increases as a function of time t according to a logarithmic function: $C_t = C_0 \cdot e^{mt}$. With C_t and C_0 being the cell concentrations at time t and 0, respectively, and m = specific growth rate. The specific growth rate is mainly dependent on algal species, light intensity and temperature (Carlsson et al., 2007).

Phase of declining growth rate: In this stage many factors such as; nutrients, light, pH, carbon dioxide or other physical and chemical factors limited the

microalgae growth and the cell division to be down slow (Carlsson et al., 2007).

Stationary phase: In the stationary phase, the cell density of microalgae is stable because the limiting factors and the growth rate are in balanced (Carlsson et al., 2007). 2.1.5. Death phase

In the final stage of microalgae, cell density decreases rapidly. That reason is the reduction of nutrient, oxygen dissolve, contamination and disorder of pH. The key to the success of microalgae production in large scale is cultured it in the exponential phase of growth and before it reach to declining growth rate phase, all of culture to be subculture (Carlsson et al., 2007).

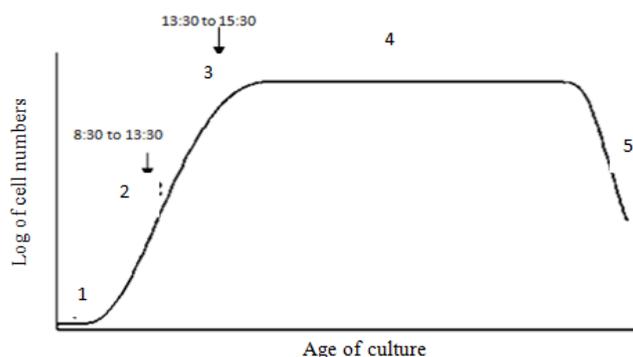


Fig.2. Growth diagram for *Chlorella vulgaris*, 1: induction phase 2: exponential phase 3: phase of declining relative growth 4: stationary phase 5: death phase.

RESULTS AND DISCUSSION

PBR report: December and November: Amounts of temperature and light (lux) in two times 8:00 AM and 15:00 PM in two months December and November have been showed in (Table.1). Temperature and light in November was higher than in December also in 15:00 PM was higher than 8:30 AM. As a result from this experiment, optimum temperature in November was 28°C and in December was 25°C. Fig (3, 4) presents the relationship between biomass and OD in November and December which indicated that the highest biomass yields were (3.0 mg/l in December) and (2.01 mg/l in November) cultured in 20-L in outdoor PBR. The biomass productivity of *C. zofingiensis* by Feng et al. (2011) (58.4 mg L⁻¹ day⁻¹)

in outdoor photobioreactor also biomass productivity of *C. vulgaris* (40 mg L⁻¹ day⁻¹) and *C. emersonii* (41 mg L⁻¹ day⁻¹) cultured in 230-L pumped tubular photobioreactor indoors reported by Scragg et al. (2002), indicating the advantage of outdoor culture (Feng et al., 2011; Scragg et al., 2002). The results of current study indicate that temperature affected the growth rate and biomass productivity (Table.1). Standard deviation and average condition factors in different times in December showed that the highest deviation was in atmosphere lux in time 8:30 (±117.7) and lowest deviation was in atmosphere temperature in time 15:00 (±1.0499) (Table 2).

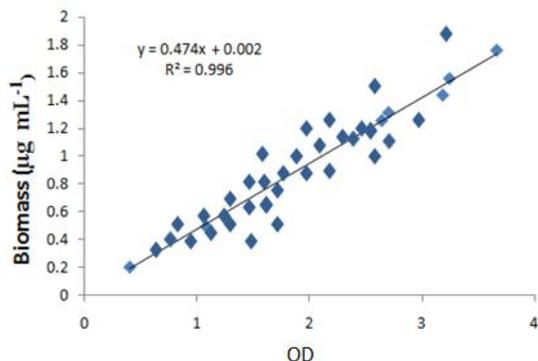


Fig.3. Relationship between Biomass and OD in photobioreactor in November (2011)

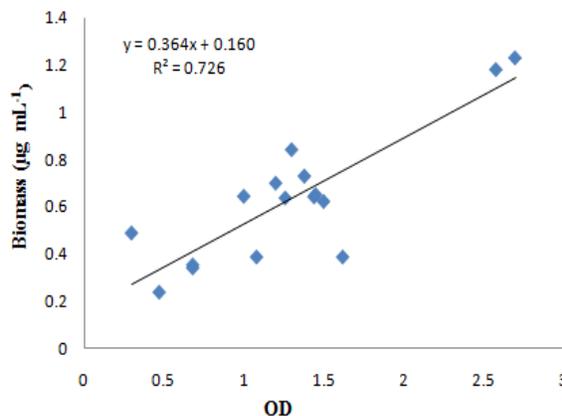


Fig.4. Relationship between OD and Biomass in photobioreactor in December (2011)

Table 1 Average of main parameters of *C. vulgaris* (Maximum★ Minimum★)

Month	Time	A Temp ^(°C)	C Temp ^(°C)	A lux
November	8:30AM	25.5	32	412
November	8:30AM	20.5	26	225
November	15:00PM	28	42	253
November	15:00PM	25	36	184
December	8:30AM	24	27.5	550
December	8:30AM	15.5	17.5	130
December	15:00PM	25	40.5	272
December	15:00PM	22	33.5	92

Table 2: Standard Deviation and Average condition factors in different time in December (2011)

Atmosphere temperature in time 8:30	Culture temperature in time 8:30	Atmosphere temperature in time 15:00	Culture temperature in time 15:00	Atmosphere Lux in time 8:30	Atmosphere Lux in time 15:00
18.97 ± 4.744	22.625 ± 4.706	24.066 ± 1.0499	36.946 ± 2.693	247.26 ± 117.7	207.6 ± 45.017

Fig.5 shows the relationship between lux and average OD in time 11:30 in December. The OD indicated that the best growth of *C. vulgaris* was obtained in lux 650 and also the optical density increased with addition amount of lux. The relationship between lux and atmosphere temperature in November has been shown in (Fig.6). Results indicated that the lux in time 8:30 was higher than in time 13:30 but the temperature in time 13:30 was higher than in time 8:30. The effect of the unfavorable conditions on biological processes is generally dependent on time. Over a range of moderate temperatures and at low to medium light intensities (relative to the normal range of the plant species) the rate of photosynthesis increases as the intensity increases and is relatively independent of temperature. High temperature changes prevented the growth of algae and caused to lower biomass

(Coutteau, 1996). Illman et al. (2000) and Carlsson et al. (2007) reported that optimum temperature for growth of *Chlorella* was at 30°C-35°C.

Bucket report: March and April: The intermediate culture was maintained in buckets as indoor and outdoor cultures. Indoor culture was placed inside the culture chamber under controlled light intensity and temperature. Outdoor culture was placed when light intensity was min/max (25/71.6) Klux and temperature was min/max (25/33). The pH was adjusted to 7.0 with 5-10% phosphoric acid for indoor and with CO₂ gas for outdoor cultures. Initial and final OD for indoor cultures was 0.25 and 0.51 µg/ml⁻¹, respectively but for outdoor cultures, they were 0.32 and 0.62 µg/ml⁻¹ respectively(Fig.7).

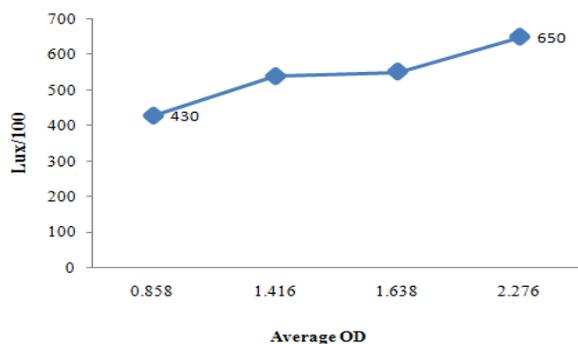


Fig.5. Relationship between Lux and Average OD in time 11:30 in December (2011)

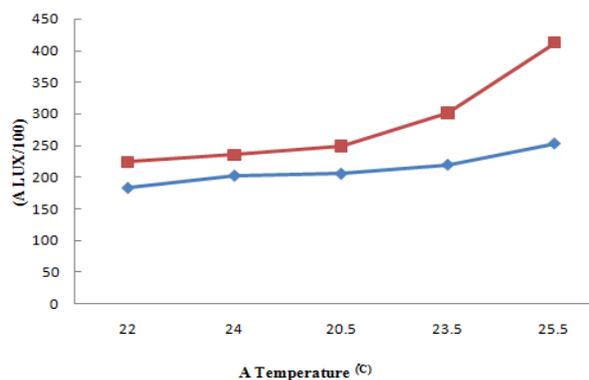


Fig.6. Relationship between Lux and Atmosphere temperature in November (2011) (Time 8:30 ◆ Time 13:30 ◆)

Tub report: March and April During the months of March and April, the culture was good and without any contamination. *C. vulgaris* cells were small in size. PH of the culture was adjusted with CO₂ gas and maintained in 6.8-7.0. In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of pH up to 9 during algal growth. Initial average OD of the culture was 0.289, biomass 0.2718g/l. Final average OD of the culture was 1.68, biomass 0.9877g/l. Min/Max Culture temperature was 18 °C- 30 °C. Min/max-Light intensity (Klux) was 9.7/89 (Fig.8). The results of Feng et al (2011) on the cultivation of *C. zofingiensis* in the autumn compared the spring showed that the cells in the spring reached higher l max (day₁) and biomass productivity and these differences could be under the influence by the differences in temperature and light intensity during the two seasons (Carlsson et al., 2007).

Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are lethal for a number of species (Barsanti and Gualtieri, 2014). As with all plants, microalgae photosynthesize capable converted inorganic carbon into organic matter (Barsanti and Gualtieri, 2014). Light is the source of energy and light intensity plays an important role that requirement to light substantially dependent on the culture depth and the density of the algal culture (Barsanti and Gualtieri, 2014). In this study at time 8:30 to 13:30 *C. vulgaris* were in exponential phase and were in phase of defining relative growth at time 13:30 to 15:30 (Fig.2). *C. vulgaris* showed different growing

behaviors at the various amounts of light and at the different temperatures. Significant increases in biomass productivities have been achieved in enclosed PBR with the use of a powerful light source and efficient light distribution (Miao and Wu, 2006). Many factors that influenced the growth of *C. vulgaris* were clarity of the water, time of day, effective range of sunlight, shape, color or transparency of the container, wall thickness and size of the container. Clear transparent PBR can be effective in absorption of visible light (Lee and Palsson, 1994). The PBRs is more expensive compare than open ponds but more harvested biomass (Chisti, 2007). The production of microalgae biomass for biodiesel goal showed that the Raceway ponds cost less money than PBRs, but the productivity of biomass is also lower than PBRs (Sheehan et al., 1998). In present experiment the growth and biomass in PBRs was higher than of bucket and tub, however the atmosphere temperature in December to November was lower than April to March in Qeshm Island. PBRs have recently attracted a considerable attention for the production for special applications. For high-density cultures, the optimization of environmental factors including light delivery, CO₂ and O₂ in the PBRs have been explored (Lee and Palsson, 1994). PBR that can be installed in areas that do not require flat land thus for some of aquaculture goal in large scale it is a good choice. For production of high-value microalgae products, light intensity and photoperiod are most important factors for growth rate and biomass composition (Khoeyi et al., 2012).

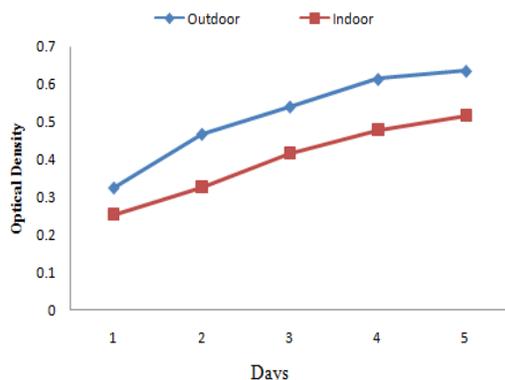


Fig.7. Optical density in different days in indoor and outdoor conditions in March and April (2011)

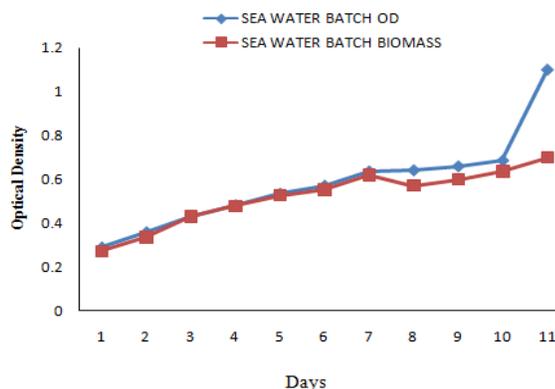


Fig.8. Optical density and Biomass in different days in SEA WATER BATCH in March and April (2011)

Conclusion: The results of current study indicated that the algae biomass production of *C. vulgaris* in PBRs was more than of bucket and tub. Also growth algae in outdoor PBR are relatively inexpensive, but are only suitable for a few, fast-growing. Outdoor production in PBRs has often unpredictable culture crashes caused by changes in weather, sunlight or rainfall. Finally, this fact is noteworthy that in outdoor conditions, temperatures and light have important role in growth of *C. vulgaris* in this study. The using of ways with lowest cost and lowest needed mineral, elected a suitable place for outdoor photobioreactors, tub and pond aspect of enough light, temperature and lowest contamination are a necessary for better growth *C. vulgaris* and highest biomass. It recommended that in an industrial scale the used of balcony water of harvesting *C. vulgaris* can be a good idea for irrigation of flowers and trees in the factory.

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