

Effect of Pigment Composition of *Porphyridium Cruentum* as Continuously Culture Method in Industrial Scale Tubular Photobioreactor

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Abstract—Since the beginning of the use of microalgal biotechnology, photobioreactors have been designed in different types and shapes. Tubular systems are the most widely used commercial systems and typically designed to use sunlight. Microalgal valuable natural products are used as animal and human food sources, pharmaceuticals and medicines. The unicellular red alga *Porphyridium cruentum* is a member of the Rodophyta, and their biochemical composition show that it is rich in many important compounds, i.e. protein, carbohydrate, lipids and carotenes. This study aims to describe a dynamic model of a bioreactor conceived for a continuous mass microalgal culture and to detect optimum drying temperature for valuable pigment composition. The *P. cruentum* were cultured in an experimental tubular photobioreactors as continuously culture method. The cell density of *P. cruentum* was held around 25.1×10^6 cells mL⁻¹ after the 9th day until the end of experiment with an average harvesting of 10%. As a result of this study, the biomass concentration spanned throughout the entire growth period and during the culture period of 45 days, totally 2250 L of *P. cruentum* was harvested as liquid biomass. Additionally, highest total β -carotene and chlorophyll a amount was measured as 415.88 ± 17.95 μ g/g and 1513.12 ± 61.78 μ g/g, respectively when the drying temperature was arranged to 180°C at spray dryer. The results of the present study indicate that outdoor tubular photobioreactor systems can be used for growing microalgae and provide many advantages when

compared with open systems. Since the best quality of *P. cruentum* biomass can be obtained at drying temperature of 180°C.

Index Terms—photobioreactor, *Porphyridium cruentum*, outdoor culture, pigment, drying temperature

I. INTRODUCTION

Aquaculture is a fast developing area and microalgal culture is a key procedure in fish hatcheries in terms of the rearing of marine fish larvae. They are an indispensable food source for all growth stages of bivalves and for larvae of some crustaceans and fish species in aquaculture as used directly in larval tanks. In this aquaculture food chain, important nutrients from microalgae are transferred to higher trophic levels via intermediary zooplankton [1], [2]. Composition of microalgae, particularly their gross composition and fatty acid content [3] are alterable significantly through culture conditions, especially depending on temperature and light conditions [4], [5]. In particular, the amount of high quality microalgae available in hatcheries is limited due to culture conditions [6].

Microalgae culture systems must be optimized and several pending problems need to be solved. Since the beginning of the use of microalgal biotechnology,

photobioreactors have been designed in different types and shapes. Outdoor tubular and flat photobioreactors are the most popular choices, due to free light source [7]. From a commercial point of view, a photobioreactor must have as many of the following characteristics as possible: large productive area, high volumetric productivity, inexpensive, easy to control and reliability [8]. The tubular systems are typically designed to use sunlight [9], [10] but they may also be designed based on artificial light sources. The main disadvantages of tubular systems are; concerns about relatively high space requirements, high light energy, cleaning problems and low efficiency in terms of mass production per unit of space [10]. Indeed, tubular systems are also far from being cheap. Considering the wide usage of microalgae especially in the aquaculture, elimination and/or reduction of such disadvantages is of paramount importance.

The unicellular red alga *Porphyridium cruentum* is a member of the Rhodophyta, order of Porphyridiales. It can be found in sea water and in humid soils. The spherical *P. cruentum* cells lack of a cell-wall. Their diameter range between 4 and 9 μm . Cells of *P. cruentum* can be solitary or massed together into irregular colonies held in the mucilage liquid [11].

Certain microalgal cell metabolites are usually produced during stationary growth phase of a culture called as secondary metabolites. They constitute some of the most important biotechnological products for humankind. A multitude of substances are involved, such as organic acids, carbohydrates, amino acids and peptides, vitamins, growth substances, antibiotics, enzymes and toxic compounds [7]-[12]. The findings about biochemical composition of *P. cruentum* show that it is rich in many important compounds, i.e. the protein content (28-39 % of dry weight) and the carbohydrate concentration [40-57 % of dry weight) and the total lipids [9]-[14] % of dry weight) and its biomass contains tocopherol, vitamin K and large amounts of carotenes [12].

Microalgae grow on nearly every biotope because of their ecological diversity and their physiological adaptation [13]. 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 a bioreactor conceived for a continuous mass microalgal culture based on physiochemical and biological principles (i.e. growth medium, optimum pH for the marine hatchery).

II. MATERIAL AND METHODS

P. cruentum was obtained from Microalgal Biotechnology Laboratory at Ben-Gurion University, Israel. *P. cruentum* were grown in laboratory 10L as a batch culture at 18 °C. The alga cultured in enriched artificial seawater (2.8 gL⁻¹ NaCl) with Porphyridium medium [11]. The medium has a 10.4 x 10⁻³ M concentration of nitrogen as a nitrogen source (KNO₃) and 5.5 x 10⁻⁴ M concentration of phosphate as phosphate source (KH₂PO₄).

The experiments were performed in a tubular photobioreactor which located in an aquaculture hatchery facility in Turkey (Akvatek Company), as shown in Figure 1. The tubular photobioreactor that was wound on a rigid vertical structure, 6 m in length, 0.2 m width and 2.65 m height that was divided into two parts, a solar receiver with a degasser and a cooler tank. The solar receiver was made of transparent plexiglas tubes and consisted of 240 m long tubes with an internal diameter of 4.6 cm and 0.2 cm wall thickness. 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 solar receiver. The microalgae 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 at couple locations which are outlet of the tube and the degasser tank with a Seko PR40 pH/redox and conductivity meter (Italy). This device was connected with solenoid valve for culture pH which was controlled automatically by injection of pure industrial grade CO₂ gas at 5 L min⁻¹. These data were also collected by a data acquisition device.



Figure1. The tubular photobioreactor system

Culture was kept illuminated with 36 fluorescent lamps (Sylvania Gro-lux 48" / 40 Watt Wide Spectrum Fluorescent Tube-T12) and 3 halogen lamps (Philips T3 150Watt) that gave an illumination of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Li-Core 195) at the surface of the tubing. These 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 as F/2 medium [14] was added daily. All cultures were maintained at 28 gL⁻¹ salinity and temperature of 20 ± 1 °C under 24 h light regime.

Cell density was measured directly under a light microscope using a 0.1-mm-deep Improved Neubauer hemocytometer and instantaneous growth rates (μ) were calculated with this equation:

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t - t_0} \quad (1)$$

Where N_t is biomass at time (t) and N_0 is the beginning biomass at time t_0 .

Growth and productivity were estimated by daily measurement of biomass concentration as reflected in dry weight. The dry mass of algal specie *P. cruentum* was determined manually by taking samples from tubular photobioreactor after specified period. A filter paper (0.45 μm , Whatman GFC, Germany) was utilized to determine the dry biomass concentration, which was previously dried in an oven at 105 °C for one hour and weighed for its initial weight. The filter papers were dried in the lab oven after filtration at 105 °C for about 4 hours. Final weight was determined by cooling the filter paper at room temperature. After filtration and before filtration difference in weights divided by the filtered sample volume provided the dry mass concentration. Total carotenoids and chlorophyll a were measured through the spectrophotometer method which is consist of extraction with in 95% methanol and measuring with UV spectrophotometer (Hitachi U-2010 Spectrophotometer, Japan). Chlorophyll concentration was measured at 665 nm and its quantity was calculated using a specific absorption coefficient of 13.9 [15]. Carotenoids concentration was measured at 480 nm and calculated on the basis of a specific absorption coefficient of 4.5 [15].

$$C_{\text{carot}} (\mu\text{g ml}^{-1}) = 4.5 A_{480} \quad (2)$$

Where A_{480} is the absorbance at 480 nm.

$$C_{\text{chlor}} (\mu\text{g ml}^{-1}) = 13.9 A_{665} \quad (3)$$

Where A_{665} is the absorbance at 665 nm.

Daily liquid biomass was taken from the culture of tubular photobioreactor system according to dilution ratio. The liquid biomass was concentrated as 20 % dry weight with disc separator (GEA Westfalia Separator, Germany). The concentrated liquid biomass was dried at 170°C, 180°C and 190°C with Spray-Dryer.

III. RESULTS

Further perspective of mass culture of microalgae will have required closed system because the microalgae must be grown under conditions that are free from potential contaminants. 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. No contamination by protozoa or other microalgae was observed in the tubular system.

Since the pH value is very sensitive to changes in other variables such as total inorganic carbon, some disturbances can appear due to inorganic carbon

concentration added into the culture medium dilution during the operation in continuous mode. In this experiment, the culture pH was maintained at 7.50 ± 0.2 by adding pure CO_2 regulated through a pH-stat system. At low cell density, the CO_2 consumption ratio was 0.4 Lh^{-1} of CO_2 , while cell density was increased to above $20 \times 10^6 \text{ cell mL}^{-1}$, CO_2 demand was increased to 1.0 Lh^{-1} .

At the beginning, light intensity was adjusted at lower level to prevent any photo-inhibition phenomena on the low cell density inoculum. Light intensity was arranged as $96 \mu\text{molm}^{-2}\text{s}^{-1}$ at the surface of the tubing with activating fluorescent lamps until 7th day. Illumination was increased to $200 \mu\text{molm}^{-2}\text{s}^{-1}$ via activation of all lights after the cell density was increased 4 times at the 9th day.

A proper reactor design is required to obtain maximum cell mass. Cell density was measured three times a day by directly counting with hemocytometer. At the same time, contamination was checked daily through visual observation. The variation of cell density of *P. cruentum* culture under the artificial light source is shown in Figure 2. The cell density of *P. cruentum* increased rapidly from 1.4 to $26.2 \times 10^6 \text{ cells mL}^{-1}$ on day 11 without any apparent log phase, and maximum specific growth rate was recorded as 0.70 day^{-1} . In this experiment, a semicontinuous (daily) harvesting regimen was adopted to maintain the preset cell concentration. When the harvest amount was exceeded by more than 10%, decrease in the cell number was determined. After the cell number declined, the harvest rate reduced to less than 10%, allowing the culture density to reach the target amount (Figure 2). The daily harvest rates have been altered for both persistence of the culture and protection of cells from degradation. The average culture harvest rate was calculated as 10.2%. So that, the biomass concentration spanned through the entire growth period.

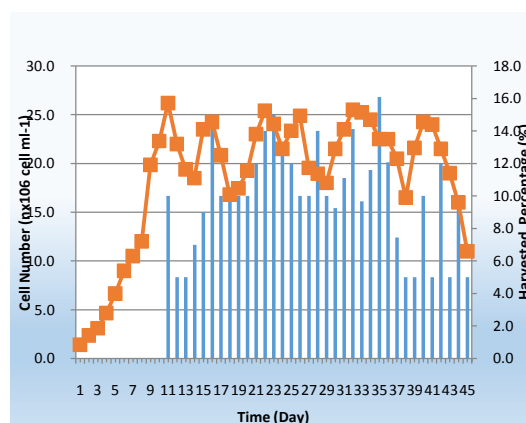


Figure2. The variation in cell density and harvested percentage of *P. cruentum* maintained at artificial light source. Line is indicated cell numbers ($\times 10^6 \text{ cell ml}^{-1}$), bar is indicated percentage of harvested (%).

As a result of this study, the biomass concentration spanned throughout the entire growth period and during the culture period of 45 days, totally 2250 L of *P. cruentum* was harvested as liquid biomass.

Chlorophyll a and total carotenoids contents were affected by drying temperature 170°C, 180°C and 190°C.

Total β -carotene values were recorded to $415.88 \pm 17.95 \mu\text{g/g}$ at 180°C , which was higher than that of the other drying temperatures. The highest value of chlorophyll a were $1513.12 \pm 61.78 \mu\text{g/g}$ when drying temperature was 180°C .

IV. DISCUSSION

Microalgal biotechnology attracts more attention from day to day 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 limits the usage of microalgae.

When the cell population density is too low, photodamage may cause the collapse of culture. Additionally, the culture being exposed to low growth irradiance or to strong light often results in photodamage in several algal species [16]. In this study, during low light intensity ($96 \mu\text{molm}^{-2}\text{s}^{-1}$) and high light intensity ($200 \mu\text{molm}^{-2}\text{s}^{-1}$), the estimated maximum specific growth rates were 0.70 d^{-1} and 0.34 d^{-1} , respectively. In this work, the light limitation was avoided by changing the intensity of the light.

Light intensity bonded with culture depth and intensity. This relation must be considered linearly. In this study, 4.6 cm diameter tubes were used with continuous illumination on both sides. The light regime prevailing in association with the narrower light-paths (less than 10 cm) could not be effective for the slow growing of *Nannochloropsis sp.* cells [15]. Photoinhibition was not detected when considering the increase in biomass concentration. This study was completed under artificial light conditions, since direct sunlight may cause inhibition on the microalgae culture. Furthermore, artificial lights allow for controlling the illumination constantly, without any dependence on environmental conditions.

V. CONCLUSION

Controlling of the photoperiod regime and temperature has been used to alter the spawning time and maturation successfully in hatcheries. Modern aquaculture provides 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 become much more 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 controlling 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 *P. cruentum* are subject to environmental conditions in each season.

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