

STUDY OF SCENEDESMUS ALGAE GROWTH IN A SPLIT-COLUMN AIR-LIFT PHOTOBIOREACTOR

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ABSTRACT

The growth of Scenedesmus algae, cultivated in a split-column airlift bioreactor under three different light intensities (94, 187, 468 $\mu E / m^2 \cdot s$) and two values of superficial gas velocities (0.3, 1 cm/s), was investigated. The physical properties of the culture medium were monitored by various analytical methods (optical density, chlorophyll concentration). Samples were taken twice a day (10:00 AM and 5:00 PM) for cell count and optical density measurements. The dynamic growth rate of the algae was studied using the integrated three-state fluid dynamics model developed by Eilers and Peeters (1988). The kinetic parameters for the system under defined light/dark cycles were evaluated. The obtained results indicate that the growth rate and chlorophyll content of the Scenedesmus algae directly proportional to light intensity and superficial gas velocity.

Keywords: Algae growth, Photobioreactor, biomass production, dynamic growth rate

دراسة نمو طحالب Scenedesmus باستخدام مفاعل بيو ضوئي ذو الرفع الهوائي الانقسامي

المخلص:

تم دراسة نمو طحالب Scenedesmus باستخدام مفاعل بيو ضوئي من نوع عمود الرفع الهوائي الانقسامي تحت ظروف مختلفة من الشدة الضوئية (94, 187, 468 $\mu E / m^2 \cdot s$) وسرع غاز سطحية (0.3, 1 cm/s). تم رصد وتقويم الخصائص الفيزيائية للوسط باستخدام طرق تحليلية مختلفة (الكثافة الضوئية وتركيز الكلوروفيل). تؤخذ عينات الفحص مرتين يوميا (10:00 صباحا و 5:00 مساء) لأغراض قياسات الكثافة الضوئية وعد الخلايا. تم دراسة معدل النمو الحيوي لطحالب Scenedesmus باستخدام موديل ديناميكية الاطوار الثلاثة التكاملية الذي طور بواسطة الباحثين Eilers and Peeters (1988). اظهرت النتائج ان معدل النمو لطحالب Scenedesmus يتناسب طرديا مع الكثافة الضوئية لمصدر الاضاءة وسرعة الغاز السطحية.

INTRODUCTION

Microalgae can be cultivated in open systems (ponds) or in controlled closed systems (photobioreactors). A photobioreactor can be described as an enclosed, illuminated culture vessel designed for controlled biomass production. Photobioreactors, despite their costs, have several major advantages over open systems. Photobioreactors minimize contamination, and offer better control over conditions such as pH, temperature, light, and CO₂ concentration.

The microalgae biomass yield, and consequent hydrocarbon production, is many times superior to that of higher plants due to the shorter algal life cycle and their more efficient growth. These organisms are not only excellent sources for biofuels (yielding ~50 times more biodiesel than soybeans per acre) due to the high lipid content (up to 40 - 80%) of some strains, but they consume CO₂ and NO_x while abating environmental pollution (processing wastewater as a nutrient source while producing O₂), and the biomass can be used in pharmaceutical products, for food additives, aquaculture, single cell proteins, etc. (Becker, 1994; Vonshak & Guy, 1992; Kulick, 1995). Although research and development to advance these potential uses has grown in recent years, the commercialization of microalgae technologies use for biofuels and bio-based chemicals production and beneficial CO₂ and waste water treatment is still in its early stages due to the complexity of the algae culturing processes and the lack of understanding. As one scales-up in size, problems become more related to engineering than biology, and the hydrodynamics and transport characteristics of the media, reactor and process play a greater role (Olaizola, 2000). The availability, intensity and duration of the light exposure can be major factors controlling productivity and hence the efficiency of CO₂ fixation (Lee and Low, 1992; Merchuk et al., 1998). The growth of the strain *Dunaliella parva* is stimulated when the provision of light is interspersed with periods of darkness (Oren and Shilo, 1982). Those previous results confirm that only the Light Harvesting (LH) phase requires light, the rest of the photosynthetic process does not. However, present models succeeded to predict algae growth in PBRs, relies on volume average irradiance (*I_{av}*), and estimate specific photosynthetic growth rates (μ) by empirical correlations that relate μ with *I_{av}* (Aiba, 1982; Evers, 1991; Rorrer et al., 1999; Molina Grima et al., 1996). These models are inherently unable to consider light gradients, light fluctuation whether motion or source induced, photoinhibition and photolimitation and the effects of fluid movement. To overcome such shortcomings, Eilers and Peeters (1988) proposed a mechanistic dynamic growth model which has been extended by Wu and Merchuk (2001) to include a maintenance constant, *M_e*, to account for the cellular damage as a function of the shear stress. Luo (2005) and Luo and Al-Dahhan (2011) demonstrated that the apparent viscosity of the media measured during the algae growth changes with time, but unfortunately their work did not account for such changes. Based on Luo's (2005) measurements, it has been found that the growth of microalgae in split-column airlift photobioreactor outperforms draft tube air-lift and bubble columns bioreactors. Therefore, split-column air-lift photobioreactor was used to further advance the development of the new multi-scale modeling approach.

The aim of this study is to use a split-column air-lift photobioreactor for culturing a *Scenedesmus* Sp. algae under two superficial gas velocities (0.3, 1 cm/s) and three irradiance intensities (94, 187, 468 $\mu E / m^2 .s$). The growth rate of the cultured algae is then estimated based on optical density and cell count measurements.

DYNAMIC GROWTH RATE MODEL FOR PHOTOBIOREACTOR PERFORMANCE

The dynamic growth rate model developed by Luo and Al- Dahhan (2004) integrates the physiology of photosynthesis and microorganism growth, the flow dynamics, and the irradiance distribution within the reactors for photobioreactor performance evaluation. This model uses the three state photosynthesis model proposed by Eilers and Peeters (1988) to describe the photosynthetic kinetics as shown in Figure (1).

Wu and Merchuk, 2004 suggested a model to calculate the residence time of an algae cell inside each part of the draft tube photobioreactor (the riser, the downcomer and the separator) based on overall gas holdup. The model concluded what is called the cyclic light history of cells in the draft tube photobioreactor as shown in Figure (2). However, Albdiri et al, 2015 showed that overall gas holdup cannot appropriately represent the dynamic behavior of the split-column airlift

photobioreactor and local gas holdup considerably varies from riser to downcomer and from the bottom to the top of the column as well.

Unfortunately the cyclic light history model (light/dark) is not applicable for the split-column airlift photobioreactor. Both riser and downcomer experience the same light exposure. Hence, light/dark cycle was performed through setting the lighting to rotating 6-hour periods of light and dark as shown in **Figure (3)**.

MATERIALS AND METHODS

- The microalgae

Secendesmus Sp. UTEX 1590 was the microalgae used. The culture was obtained from the collection of the University of Texas, Austin, USA. The inoculum for the photobioreactors was grown indoors under artificial light. Nutrient requirements are salts, nitrates, phosphorus, carbon source, and air. Ideally, food should contain minimal carbon content to allow for carbon dioxide (no more than 3% for this strain) to be the primary carbon source. Current food source is algal supplement Proline F1/F2 fed 1mL/gal every Monday, Wednesday, and Friday while not on CO₂ supplement and 1-2 times a week while on CO₂ feed. Tanks are stirred manually at least every other day to ensure the algae doesn't settle out and to make diffusion of nutrients throughout the system as even as possible. Air flow rate was set to 2500 mL/min with adjustments being made to incorporate CO₂ doping of the air feed of percentages between 1.5 and 3 percent for this particular strain; however, the air feed was set to 4000 mL/min when no CO₂ doping is present to allow CO₂ from the natural filtered air to be sufficient to support algal growth. Care was taken to monitor pH since the incorporation of CO₂ in the water lowers pH outside of long-term tolerance ranges. pH tolerance range for long term is 6.8 to 8.2. The best growth is achieved when pH is maintained between 7.0 and 7.5.

Lighting source is 3 x 6500k T5 fluorescent lamps placed 12-18 inches above growth tanks and approximately 3 ins apart to ensure even lighting across the growth tanks. The light timing is set to match the cycles simulated in reactor. Two-week lead-time was adjusted to the light/dark ratio before tests been run in the reactor in order to achieve consistent results. The lighting was set to rotating 6-hour periods of light and dark.

- The photobioreactor and culture medium

The split-column airlift photobioreactor used in this study is shown schematically in **Figure (4)**. The column was made from Plexiglas with an inner diameter of 13 cm and height of 150 cm. A Plexiglas plate divides the reactor into two zones of equal cross-sectional areas. The liquid volume of the reactor is 15 liters. The top clearance (the distance from the top edge of the splitting plate to the static liquid level) was 3 cm while the bottom clearance was 5 cm. At the bottom of the riser section of the photobioreactor, a sparger **Figure (4-b)** was installed at the center of the riser. The column has 5 ports for measurement (P1 through P5 in the figure) on both sides (riser and downcomer). These ports are at heights of 29.90cm, 52.00 cm, 76.05cm, and 100.1 cm (equivalent to 4.6R, 8R, 11.7R, 15.4R) respectively. Before each experiment, the whole column was carefully washed with soap water and thoroughly rinsed with deionized water. A cotton plug was provided to purify the compressed air before it enters the column through the ring sparger, and the column was loosely covered by a lid. The compressed air, enriched with 3% CO₂ (Merchuk et al., 2000), was introduced into the reactor through the ring sparger, providing both carbon source and agitation for the photobioreactor. Light energy was supplied continuously by a bank of cool white Sylvania fluorescent lamps (four 40Watt lamps with an intensity of 3000 Lumen and eight 60W high output lamps with an intensity of 3281 Lumen) evenly distributed around the airlift column. Such configuration of the lamp bank, generated a fairly uniform light intensity distribution around the

illuminated column surface. The lighting was set for rotating 6-hour periods of light and dark. After the airlift column photobioreactor was inoculated with *scenedesmus sp.*, the optical density of the culture was initially very low (less than 0.01). To avoid photoinhibition and to shorten the lag time, the cells need to be adjusted to the new environment. The reactor was first run for 12 hours at low light intensity and at low superficial gas velocity (i.e., illuminated only by the room lamps without switching on any bank lamps, Photon Flux Densities (PFD) was around $26 \mu E/m^2 s$ and the superficial gas velocity was 0.3 cm/s). Then, four 40W lamps were switched on while keeping the same superficial gas velocity, giving a PFD around $275 \mu E/m^2 s$ on the illuminated surface. After the optical density of the culture reached 1.0, the number of lamps to be switched on, was increased and controlled, giving high irradiance (i.e., up to $468 \mu E/m^2 s$). Finally, to test the effect of mixing intensity on the airlift column photobioreactor performance, the superficial gas velocity was increased to 1cm/s while keep all lamps on.

ANALYTICAL METHODS

A 5 ml pipette was used for sample preparation. The pipette was carefully cleaned and sterilized before it was inserted into the top part (where mixing is perfect) of the photobioreactor for sampling. Each sample was divided into two parts; the first was usually used for optical density measurements, while the second sample was used for cell count measurements. The cuvette was cleaned and sterilized before it was used for optical density measurements, and the cell count chamber was used for the cell count measurements.

- Optical density

Optical densities of the algae samples were measured at least twice a day (10:00 AM and 5:00 PM). A spectrophotometer (UV-Visible GENESYS 10) and cuvettes with path length of 1cm were used for the measurements at wavelengths of 680, 666, 656, and 648nm. For each optical density measurement, an average of three samples was taken as the real optical density.

- Cell Number Counting

Cell numbers were counted using a counting chamber (Bright-line counting chamber, Fisher Healthcare) under a microscope (Olympus 324, Olympus Inc.) with 400× zoom.

- Concentration of Chlorophyll

The chlorophyll content was estimated by the method of Liu et al. (1981). Optical density was measured at 648, 656 and 666 by using UV-visible spectrophotometer (UV-Visible GENESYS 10). The chlorophyll a and b content was calculated using the following equations (**Maraskolhe, et al (2012)**):

$$C_a (\mu g ml^{-1}) = 13.7 A_{666 nm} - 5.76 A_{648 nm} \quad (1)$$

$$C_b (\mu g ml^{-1}) = 25.8 A_{648 nm} - 7.60 A_{666 nm} \quad (2)$$

$$C_{a+b} (\mu g ml^{-1}) = 1000 / (39.8 \times A_{656 nm}) \quad (3)$$

- pH measurements

pH measurements were carried out twice a day using HI991001 waterproof pH meter to monitor the effect of CO₂ consumption by algae culture and consequently the algae growth rate in the split-column airlift bioreactor.

RESULTS AND DISCUSSION

- Effect of superficial gas velocity on *Scenedesmus sp.* growth

The growth rate of *Scenedesmus* algae, expressed as cell number vs. cultivation time, for two different superficial gas velocities (0.3 and 1.0 cm/s) and under (94 $\mu\text{E}/\text{m}^2.\text{s}$) light intensity is shown in **Figure (5)**. It appears that the growth rates were similar during the initial phase (< 2 days) of cultivation for both superficial gas velocities. Then, the growth rate of the higher superficial gas velocity (1 cm/s) diverged from the lower superficial gas velocity (0.3 cm/s) with a 25% increase. The growth of the culture experienced a slight growth of algae. This followed the expected pattern of a lag phase, during which algae needs to become established. During the initial stage of the exponential phase of the growth of algae (days 2-4), it was observed that the growth rates under different superficial gas velocities were identical. This is due to low rate of growth (0.04 h^{-1}) as the culture was exposed to low light intensity in avoidance to photo inhibition. The divergence between the growth rates on day 4 was attributed to the effect of mixing on the growth rate of algae. The mixing enhances the chance of algae to be directly irradiated. Since the mixing rate at a superficial gas velocity of 1 cm/s is higher than that of 0.3 cm/s, the growth rate of algae will be higher as well. The growth rate at the exponential phase ranged from (0.209 h^{-1} for 0.3 cm/s to 0.285 h^{-1} for 1 cm/s). This large difference in growth rate reflects the importance of increasing the superficial gas velocity to perform better mixing and consequently higher growth rate. However, higher superficial gas velocities usually cause higher hydraulic force (shear stress) which may break microorganism cells. **Luo and Al-Dahhan (2005)** found that a superficial gas velocity of 1 cm/s is a tolerable velocity.

- Effect of light intensity on *Scenedesmus sp.* growth

Figure (6) shows the dependence of the growth rate of *Scenedesmus* algae on the light intensity provided by the bank of cool white Sylvania fluorescent lamps surrounding the photobioreactor column. During the initial phase of cultivation, the growth rate of algae was found to be similar for all light intensities (94, 187, 468 $\mu\text{E}/\text{m}^2.\text{s}$). However, during the exponential phase of the growth of *Scenedesmus* algae, the growth rate under light intensity (468 $\mu\text{E}/\text{m}^2.\text{s}$) begins to significantly depart (approximately 31%) from that of light intensities (94, 187 $\mu\text{E}/\text{m}^2.\text{s}$), whereas a slight difference in the growth rate values under 94 and 187 light intensities was noticed in favor of the light intensity 187 $\mu\text{E}/\text{m}^2.\text{s}$ (13% difference). The cultivation was carried out at a superficial velocity of (1 cm/s). The interpretation of the above large difference in the growth rate of algae under the light intensity 468 $\mu\text{E}/\text{m}^2.\text{s}$ may be attributed to the abundant light energy available for the photosynthesis of algae. This confirms the fact that the higher the light intensity, the higher penetration depth of light inside the photobioreactor at high culture density of algae. However, no photo inhibition was noticed when applying 468 $\mu\text{E}/\text{m}^2.\text{s}$ light intensity.

- Evolution of the chlorophyll content of *Scenedesmus sp.* algae

The build-up of the chlorophyll a and chlorophyll b contents of *Scenedesmus Sp.* algae is shown in **Figures (7,8)**. The results show that the evolution of both chlorophyll a and chlorophyll b follows the same trend of the evolution of the cell count of *Scenedesmus Sp.* algae shown in **Figure (5)**. However, the evolution of chlorophyll was found to be highly dependent on the light intensity. Approximately 100% increase in chlorophyll evolution was observed under higher light intensity (I = 468 $\mu\text{E}/\text{m}^2.\text{s}$). A moderate gain in chlorophyll content was observed during this study in comparison to Maraskolhe's work (2012). This may be due to lower concentration of carbon dioxide (3%) when compared to Maraskolhe's work (10-36%). However, these results are consistent with the conclusion of Lue and Al-Dahhan (2005). They concluded that split-column photobioreactor demonstrates light stress to cultured algae due to its high performance of mixing (high frequency of wall visits by the cultured algae).

CONCLUSIONS

The growth rate of *Scenedesums Sp.* algae was found to be substantially dependent on superficial gas velocity. The higher the superficial gas velocity, the higher mixing intensity and the higher growth rate of algae. However, critical shear stress of algae must be considered and not exceeded when designing for high superficial velocities. Light intensity was also found to have a direct effect on the growth rate of algae, but, the photo inhibition must be avoided when applying light source for culturing algae. Although, the growth rate of *Scenedesums* algae increases with higher superficial gas velocities, but, the chlorophyll content was found to grow at lower rate due to the light stress that may be caused by the high frequency of wall visits of the algae.

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NOTATIONS

X: biomass concentration ($\mu\text{g/ml}$)

X_1 : fractions of the reaction centers in the resting state

X_2 : fractions of the reaction centers in the activated state

X_3 : fractions of the reaction centers in the inhibited state

I: instant light intensity exposed to the cells

$\alpha, \beta, \delta, \gamma$: Photosynthetic reaction constants

C_a : chlorophyll a content

C_b : chlorophyll b content

C_{a+b} : chlorophyll a and chlorophyll b contents

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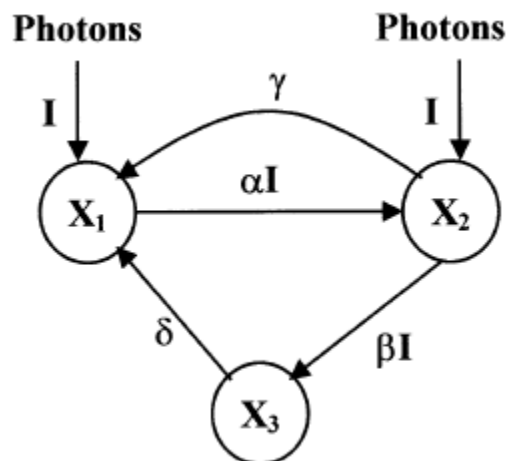


Figure (1): The Structure of the three states model proposed by Eilers and Peeters (1988) (duplicated from Wu and Merchuk, 2001). A photosynthetic reaction center in state x_1 captures a photon and passes to the reactive state, x_2 , at a rate proportional to the light intensity, I . The reaction center in state x_2 can either return to state x_1 at a constant rate, γ , or capture another photon and reaches the inhibited state x_3 . At state x_3 , the reaction center returns to state x_1 at a constant rate, δ . The chain of dark reactions is started by the direct passage of $x_2 \rightarrow x_1$.

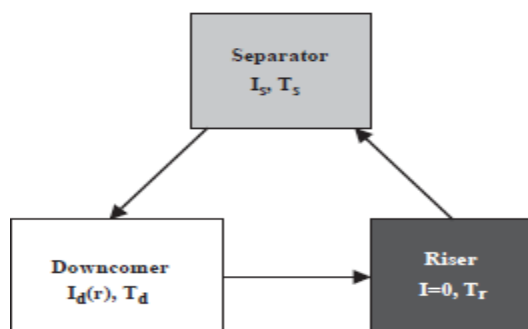


Figure (2): Cyclic light history of cells in airlift photobioreactor. (duplicated from Xiaoxi Wu and Jose C. Merchuk, 2004).

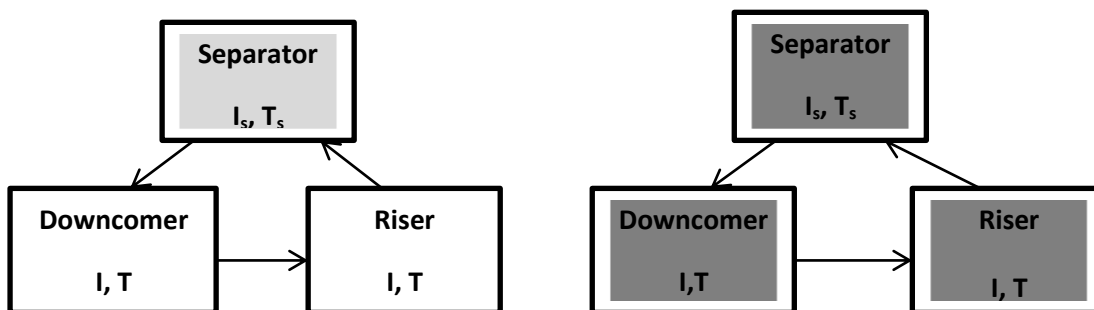


Figure (3-a): light history of cells in Split-column airlift photobioreactor 6-hours light on period.

Figure (3-b): light history of cells in Split-column airlift photobioreactor 6-hours light off period.

Figure (3): cyclic light history of cells in split-column airlift photobioreactor.

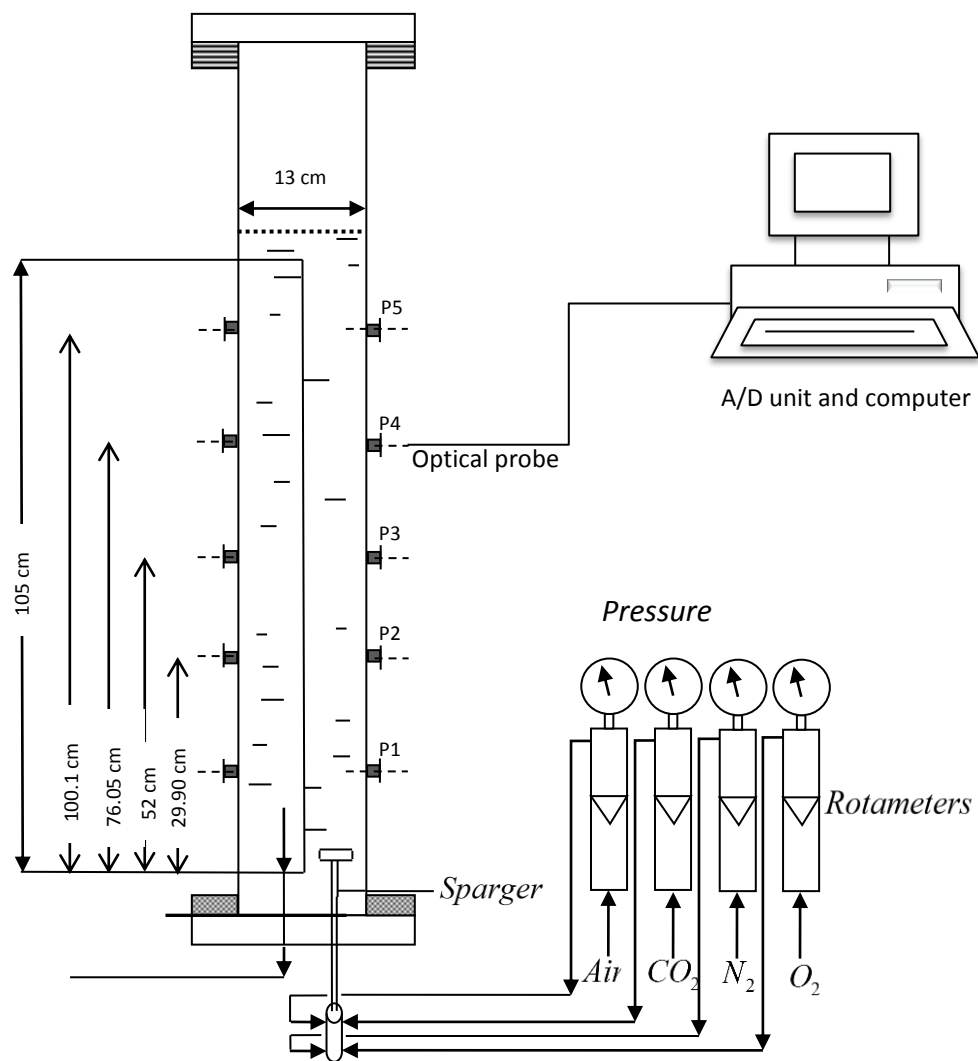


Figure (4- a): split-column (P1-5: ports 1-5)

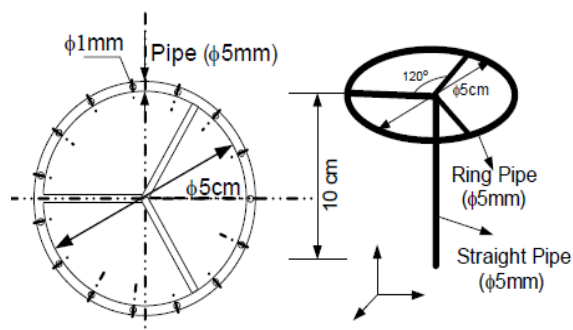


Figure (4- b): sparger

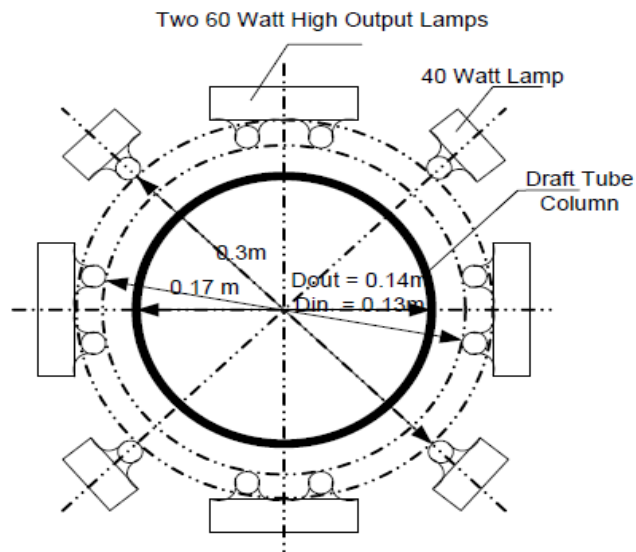


Figure (4- c): Configuration of the lamps to illuminate the reactor.

Figure (4): Schematic representation of split-column airlift bioreactor, sparger and bank of illuminating lamps.

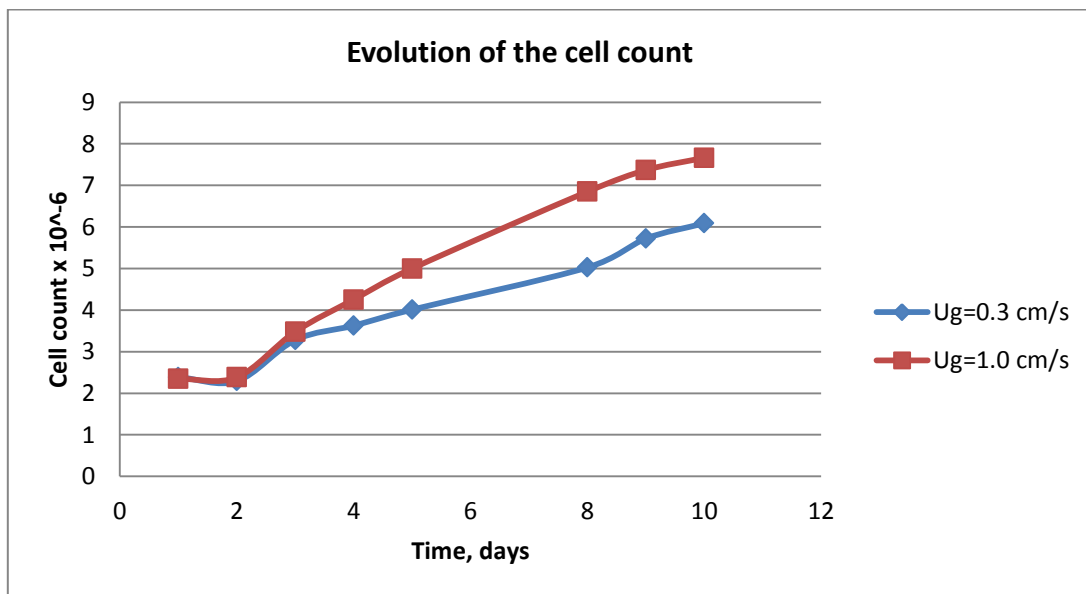


Figure (5): Effect of superficial gas velocity on the Growth of Scenedesmus Sp. algae in a split-column airlift photobioreactor.

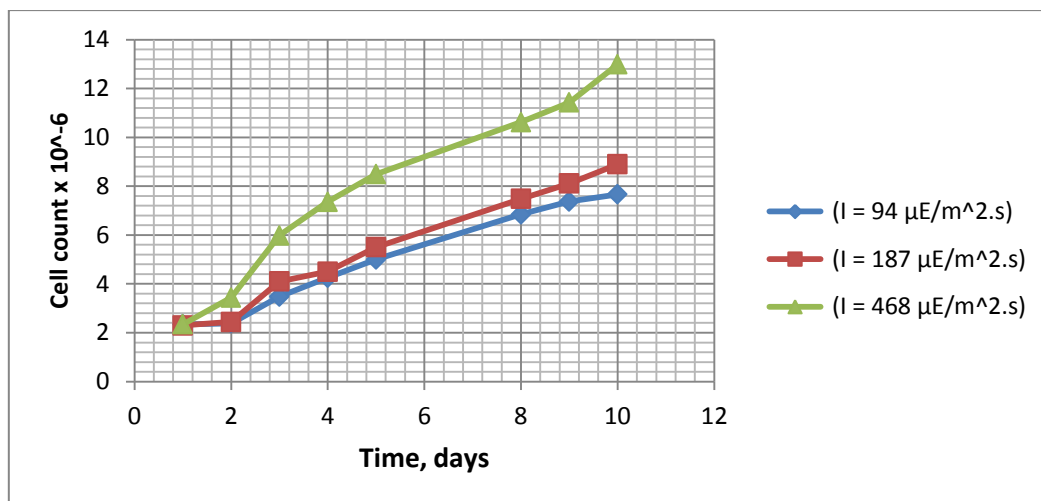


Figure (6): Effect of light intensity on the Growth of Scenedesmus Sp. algae in a split-column airlift photobioreactor (Superficial gas velocity equals to 1 cm/s).

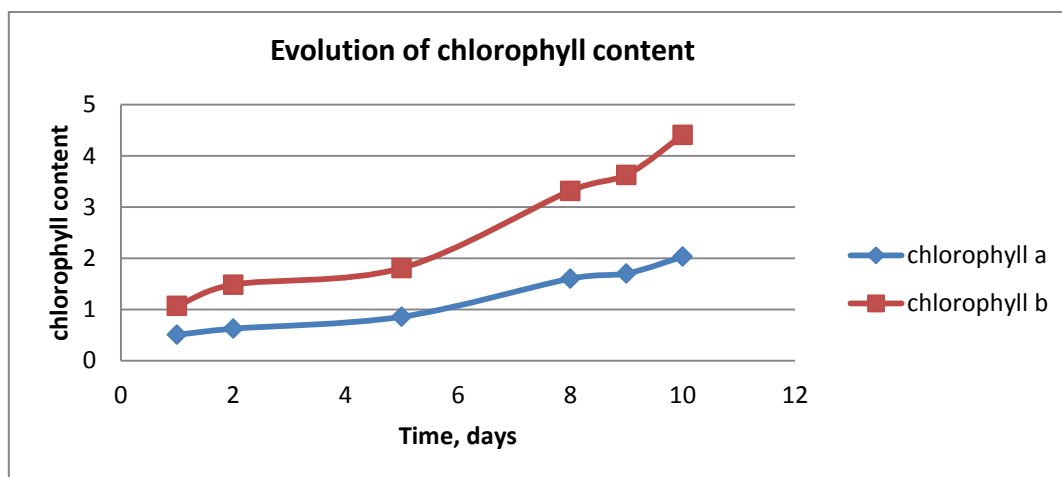


Figure (7): Evolution of the chlorophyll content of Scenedesmus Sp. algae in a split-column airlift photobioreactor (I = 94 μE/m².s and superficial gas velocity = 1 cm/s).

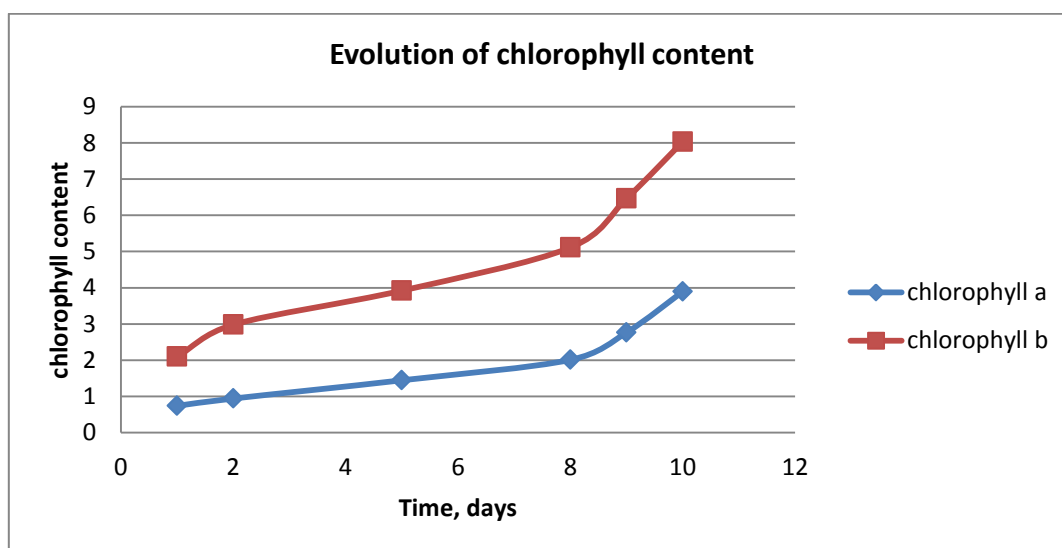


Figure (8): Evolution of the chlorophyll content of Scenedesmus Sp. algae in a split-column airlift photobioreactor (I = 468 μE/m².s and superficial gas velocity = 1 cm/s).