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An integrated photobioreactor system for the production of *Spirulina platensis*

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Abstract: In this study, a laboratory scale integrated type of a photobioreactor system was operated for the production of *Spirulina platensis*. The integrated design consisted of a tank unit for the main production site and a helical coil unit for the main illumination site. Different trials were made in order to select the best illumination mode. The best illumination mode was the sequential mode where a continuous illumination was taking place in the internally illuminated tank unit and at the same time a 12/12 h light dark cycle was going on, in the coil unit. The system maintain the stability in the pH levels and a cell concentration of 3.12 g L^{-1} on dry weight basis. After the selection of the illumination mode the best flow rate and light intensity synchronization was tested by applying various combinations of intensities and flow rates in the coil unit. The best values were obtained with the 11000 lux intensity at 7 cm sn^{-1} flow rate in the coil unit reaching a growth rate of 0.42 day. The determination of the best conditions for the working of the integrated system was one of the main objectives of this study. Also to focus on an integrated system that could be used in commercial scale, that could succeed the need of high volume production at the same time to fulfill the need of light compensating the dark regions that could happen in the big tanks like commercial fermenters with outdoor or indoor helical tubular units as photostages.

Key words: *Spirulina platensis*, photobioreactors, internally illumination, helical tubular photo bioreactors

INTRODUCTION

Microalgae are microscopic organisms, with color pigments and mostly autotrophic, living in aqueous environments. Microalgae are used in industry cultivating in photobiorector systems because of their valuable products and (Olaizola, 2003; Richmond, 2000; Tramper *et al*., 2003). In 1827 P.J. Turpin isolated *Spirulina* from a fresh water sample and Wittrock and Nordstedt reported the presence of *Spirulina* near the city of Montevideo in 1844 (Sanchez *et al*., 2003).The first unialgal cultures were achieved by Beijernick (1890) with *Chlorella vulgaris* (Borowitzka, 1999). *Spirulina* is a photosynthetic, filamentous, blue-green colored microalge which can grow rapidly. Richmond (2000) defines its structure of 1-12 μm diameter helical shape (Anderson *et al*., 2002; Delibas, 2003; Barbosa *et al*., 2003; Bosma and Wijffels, 2003).

Microalgea have close interactions with the environment that they are living because of that the parameters which are closely related to their environment have to be in optimum levels. Temperature, light, nutrients and pH are very important parameters for microalgea (Tanaka *et al*., 1995a-c; Molina *et al*., 1999)

Light is playing an important role in microalgal cultivation and growth rate of photosynthetic organisms like microalgae is closely related with the light. At the works to find optimal levels of light intensity, growth rate of microalgae increases as proportional with increasing light intensity until saturation levels. Further increase in light intensity will cause inhibition in cellular growth (Pulz, 2001; Tanaka *et al*., 1995a-c; Torzillo *et al*., 2003; Lee, 2001). Mixing is also another important parameter for microalgae cultivation and it is playing an important role in optimization of light penetration, temperature distribution and various other effects which is vital in cultivation. The mixing regime helps the cultivation medium to have enough substrate and overall homogeneity of other parameters (Becker, 1995).

Oxygen and carbon dioxide transferred in the culture is directly related for cultivations and these gases are the important effectors of cellular metabolism and growth rates. Transfer of these gases is the building block of cultivation system design and scale-up (Chisti *et al*., 2003; Chisti, 1998). Oxygen is using by cells as well as carbon dioxide. Excess or lack of these gases will cause inhibition in cultivation. Excess oxygen will cause oxidation during

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cultivation which will decrease productivity. Carbon dioxide is the main carbon sources for phototrophic cultivation and lack of carbon dioxide can cause severe problems in the culture (Becker, 1995; Cohen, 2000; Degen *et al.* 2001).

Microalgae culture system can be classified by their design and construction as well as by their circulation mechanism, high source, aeration system and interactions with their environments. As an example according to light source they can be studied as solar illuminated systems and artificial illuminated systems or according to interaction with their environment open and closed systems (Chisti *et al.*, 2003; Pulz, 2001).

The term photobioreactor is defining a closed system where the optimum environment is obtained for cultivation by controlling the system. Commercial production systems for photobioreactors that are used wide spread are; tubular, panel, airlift and agitated tank systems. Integrated systems are constructed in order to mix the advantages of different photobioreactors systems. Aim is to improve the productivity and process. They are also applicable for different nutrient modes. Also integration of photobioreactors with waste water treatment systems are used in aquaculture and agriculture farms (Apt and Behrens, 1999; Ogbonna *et al.*, 1997, 2000; Fox, 1996).

Photobioreactors and their different systems are focused on feasible microalgal production. The life of a facility will be closely related to the performance of its productivity and economy (Lee, 2001; Olaizola, 2003).

In this study an integrated photobioreactor system was used for the production of *Spirulina platensis* in laboratory scale (Öncel and Akpolat, 2004).

MATERIALS AND METHODS

Microorganism, photobioreactor (PBR), culture media and inoculation: *Spirulina platensis* was from the collection of EGE-MACC of Algae Research and Development Unit in Science and Technology Research and Application Center (EBILTEM). The origin of the strain is from Parachas lake in Peru and taken from ACMA (Association pour Combatre la Malnutrition par Algoculture) in France from Fox (1996). The culture media used was Zarrouk's medium and the applied thermal sterilization is at 120°C for 15 min (Becker, 1995; Borowitzka and Borowitzka, 1992).

PBR used has two production units (Fig. 1). The coil unit is based on the Biocoil design and integrated unit was an internally illuminated glass fermenter. The PBR parts comprise: A helical photostage made of 76 m transparent polyurethane tubing (6×8 mm) was used. The

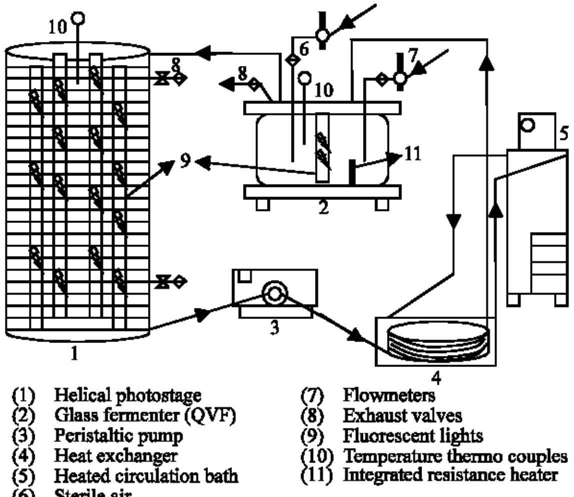

Fig. 1: Schematic diagram of the PBR system

tubing was fixed with metal wire skeleton to get a vertical cylinder of 65 cm height and 35 cm diameter (1). A glass fermenter (QVF) stainless steel top and bottom cover (inside diameter: 23 cm, height: 20 cm, thickness of glass: 1.5 cm) modified as a photobioreactor with a submerged glass tube (diameter: 2.6 cm, height: 20 cm) housing a 8 W fluorescent light (2). A peristaltic pump (Masterflex 7521-35) with rotor speed control (3). A helical photostage made of 76 m transparent polyurethane tubing (6×8 mm) was used. The tubing was fixed with metal wire skeleton to get a vertical cylinder of 65 cm height and 35 cm diameter (1). A glass fermenter (QVF) stainless steel top and bottom cover (inside diameter: 23 cm, height: 20 cm, thickness of glass: 1.5 cm) modified as a photobioreactor with a submerged glass tube (diameter: 2.6 cm, height: 20 cm) housing a 8 W fluorescent light (2).

A peristaltic pump (Masterflex 7521-35) with rotor speed control (3). A heat exchanger made by using 24 m polyurethane tubing curved as a coil submerged in a water bath with fixed temperature (4). A heated circulation bath used to fix the temperature in the PBR's (5). The sterile air was supplied 4 L min^{-1} through the 0.2 μm PTFE filters (sartorious midisart 2000) from two injection points (2 L min^{-1} from each points) over the glass tank unit (6). The flow rate of the inlet air was controlled with flowmeters (7). The exhaust valves on the coil unit used to release excess gas to prevent stress of excess gas and to provide plug flow inside. Also they were used to pump air inside the tubes during the cleaning procedure (8). Fluorescent lights were used for illumination. 18 W lights were fixed inside the coil and 8 W light put inside a glass housing and submerged inside the glass unit (9). Temperature was controlled with digital thermocouples inside the units (10). An integrated resistance heater

provided the high temperatures for sterilization procedure (11). The outer surface of both units was covered with white cardboard in order to prevent the absorption of outside light. The working volume of the system was 8 liters and it was distributed in the PBR as; 2.5 L in coil unit, 5 L in glass unit, 0.68 L in heat exchanger and 0.2 L in fittings.

The start-up culture was carried out in 250 mL Erlenmeyer flasks for 4 weeks and every week it was transferred to a fresh medium inoculated with the seed culture at 1/10 (V:V) dilution aseptically. After 4 weeks the culture was transferred at the same dilution rate to 1 L Pyrex bottles and the aeration in the bottles was fixed at 1 L min^{-1} with sterile air. The intensity on Erlenmeyer flask surface was 2000 lux and in bottles 4000 lux and temperature was 25±1°C. The light intensity was measured with a luximeter (LUTRON LX-105) and 40 W daylight florescent lamps were employed (Philips TLD/54).

Fresh medium was than inoculated with the seed culture at 10% volume. Dilution amount was controlled to have; OD 0.175-0.180 at 560 nm and a DW of average 0.1 g L^{-1} for the inoculation concentration at the start.

Culture conditions, experiments and sterilization procedure: Cultures were carried out in a two unit integrated PBR at constant temperature (32±1°C). Coil unit was illuminated with 4 daylight fluorescent lights (18 W OSRAM) and the tank unit illuminated internally (8 W OSRAM). Different illumination and circulation flow rates were tested through the experiments. Illumination mode experiments were carried out at constant 4.5 cm sec^{-1}. Experiments were:

- Continuous illumination (where all the lights were active providing 11000 lux on the coil unit and 6000 lux in the tank unit),
- Continuous subsequent illumination (where only the intensity of the coil unit was reduced to 5700 lux on the 9th day and the illumination was carried out continuously),
- Sequential illumination (where the light intensity fixed at 5700 lux on the coil unit was applied as 12/12 h light-dark cycle and the illumination in the tank unit was carried out continuously).

After the illumination mode experiments different circulation flow rates in the coil unit was tested. Circulation rate was determined by injection of a clump of *Spirulina* cells into the coil unit at different rotation speeds of the peristaltic pump and measuring of the clump movement between two points in the downstream tube. Circulation speed experiments were carried out at different light intensities at the coil unit and the illumination mode was sequential which was chosen according to the results of the previous experiments. Experiments were:

- Sequential illumination with 4.5 cm sec^{-1} flow rate (where the intensity on the coil unit was 11000 lux)
- Sequential illumination with 4.5 cm sec^{-1} flow rate (where the intensity on the coil unit was 5700 lux)
- Sequential illumination with 7 cm sec^{-1} flow rate (where the intensity on the coil unit was 11000 lux)
- Sequential illumination with 7 cm sec^{-1} flow rate (where the intensity on the coil unit was 5700 lux)
- Sequential illumination with 10 cm sec^{-1} flow rate (where the intensity on the coil unit was 11000 lux)
- Sequential illumination with 10 cm sec^{-1} flow rate (where the intensity on the coil unit was 5700 lux)

Measurements and determinations were made daily by taking 20 mL culture sample. After sample collection equal amount of fresh medium was added to the system to maintain a constant volume. Also in order to prevent evaporation losses level was controlled daily.

The PBR was chemically sterilized in a multi step procedure. First all the parts were washed with water to prevent any dust or waste. After the parts were dried they attached and fixed and PBR filled with sterile distilled water. The water was than taken out of the PBR and 1% (W:V) sodium hypochlorite solution filled in the PBR to the top. After that water was left to rest overnight. For the last step the PBR was washed with sterile distilled water for several times to remove organic and inorganic residues. At the end the system is emptied and left to dry before culture addition.

Measurements:

Dry weight, chlorophyll-a, cell count, cellular turbidity and pH determination: Dry weight was determined on triplicate samples of the cultures (5 mL each). Culture samples were washed 1/10 V:V with distilled water in order to dissolve excess salt. The water added samples were filtered through Whatmann GF/C (1.2 μm, 47 mm diameter) filters. The filtered cells were dried overnight at 105°C until constant weight (Torzilla *et al*., 2004; Vonshak, 1997).

Chlorophyll-a measurement was done in triplicates with methanol. Five milliliter sample filtered through GF/C filters and mixed with 5 mL pure methanol. The samples were later heated in 70°C water bath for 3 min. Than centrifuged for 5 min at 3500 rpm in the (Hettich universal 32R) centrifuge. Supernatant absorbance was determined at 665 and 750 nm according to pure methanol blind. Chlorophyll-a amount was calculated by 13.9 (A_{665}-A_{750}) equation in mg L^{-1} (Torzilla *et al*., 2004; Vonshak, 1997).

Cellular growth was measured daily in triplicates by cell microscopic counting using a Neubauer haemocytometer (Marienfeld, Depth 0.100 mm, 0.0025 m^2).

The cellular turbidity was determined every day by measurements of optical density at 560 nm with a JENWAY 6400 spectrophotometer (Torzilla *et al.*, 2004; Vonshak, 1997).

The pH level was measured daily with a with Sartorius (pH/ATC electrode, 3M KCl) electrode.

RESULTS

The first stage of the experiments, study focused on the illumination mode selection. When all the fluorescent lights were employed in continuous mode after day 4 the culture pH level reached to 11.12. The pH evolution showed a significant increase from 9.15 to 11.12 which caused problems in culture. The start-up values of the culture was 0.1 g L^{-1} in dry weight and at day 4 it was 1.88 g L^{-1}. After day 6 the increase in pH level reached to a maximum of 12.05 and thick foam on the culture became more dense. At the end of day 8 a settlement of the culture with a yellowish color observed. This can be indication of the fact of photolysis or the destruction of chlorophyll by photon bombardment. The yellowish color with foaming was the indication of the lysis had happened and the cell walls ruptured, expelling polysaccharides onto the medium. These symptoms of problems coincide with the symptom analysis of Fox (1996). After this stage illumination with subsequent mode was continued for the next experiments with the focus on the change in pH level.

During subsequent illumination the pH level had reached the maximum on the 9th day of the cultivation. This day was chosen for the decrease of the light intensity in the coil unit because of the previous experience with the continuous illumination mode experiments. After pH level reached to 11.21 on the 9th day the effect of the decrease showed a significant improvement in the pH level and a constant flow could be protected between levels of 10 and 10.5 till the end of the experiment (Fig. 2a).

The rise in the pH level was better controlled when the sequential illumination mode employed for the next experiment (Fig. 2a). This mode was chosen to prevent the pH increase before reaching to the high levels that can be severe to the culture which was faced during the continuous illumination and tried to be handled with subsequent illumination. With sequential mode the 12 h cycle was effective on the pH control and fixed the pH level on the 9 to 10 interval during the 17 days of cultivation. This mode was successful to control pH level even with the narrow tubing used as the photo-stage in the coil unit.

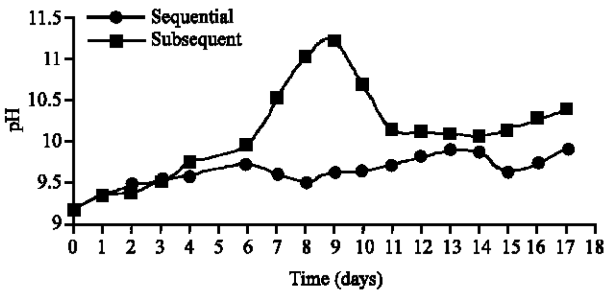

Fig. 2a: pH evolution during the cultivations with different illumination modes

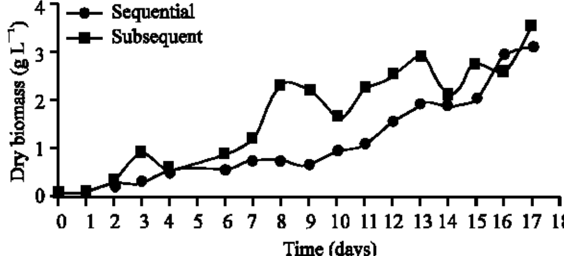

Fig. 2b: Variation of dry biomass (g L^{-1}) during the cultivations with different illumination modes

Dry biomass showed an increase till day 8. On day 9 when the pH level continued to increase indication of the high intensity that caused a stress on the culture, the dry biomass weight started to decrease. The decrease in the intensity showed its effect after day 10 with the increase trend on the dry weight (Fig. 2b). The dry weight reached 3.55 g L^{-1} on day 17.

During the sequential illumination mode the dry weight had followed a continuous increase through the cultivation time and reached to 3.12 g L^{-1}. Even if this value seemed to be less than the value reached with the subsequent illumination the loss in the dry weight was not a problem like in the other mode where a loss of biomass was observed through the cultivation because of the instability of the culture. The different trend for the two different modes could be compared from Fig. 2b. The first 8 days of cultivation when compared according to the growth rates subsequent mode lead to 0.41 day and sequential mode to a 0.36 day. But after day 8 subsequent mode caused biomass loss in the following two days until the decrease in intensity showed its effect.

Optical density varied very similar to the dry weight of the culture. Again the OD values were a little high with the subsequent illumination mode compared to the sequential illumination mode. OD value reached to 6.67 with subsequent mode while reached to 5.92 with sequential mode. But it was also worth to mention that the increase in the sequential mode was more stable compared to the other illumination mode (Fig. 3a).

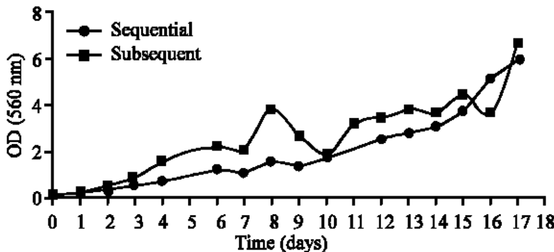

Fig. 3a: Variation of optical density with different illumination modes

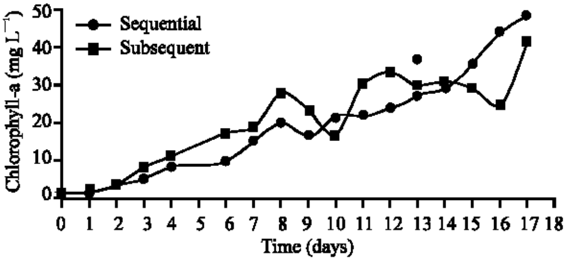

Fig. 3b: Variation of chlorophyll-a concentration with different illumination modes

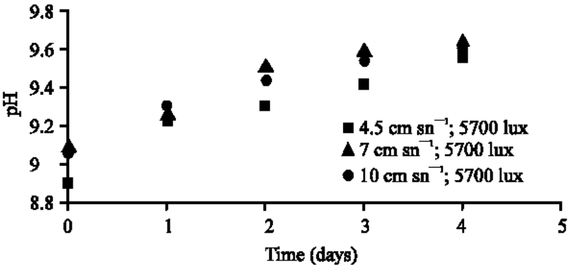

Fig. 4a: pH evolution during the cultivations with different flow rates at 5700 lux

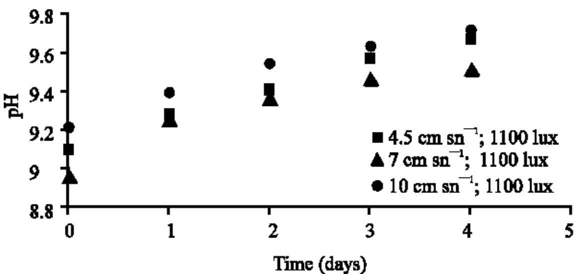

Fig. 4b: pH evolution during the cultivations with different flow rates at 11000 lux

When the chlorophyll-a concentrations were estimated the effect of the decrease in the intensity after day 9 showed a decrease at the first stage but later an increasing trend could be cached. The chlorophyll-a concentration had reached a value of 41.4 mg L^{-1} at the end of the cultivation with subsequent mode (Fig. 3b).

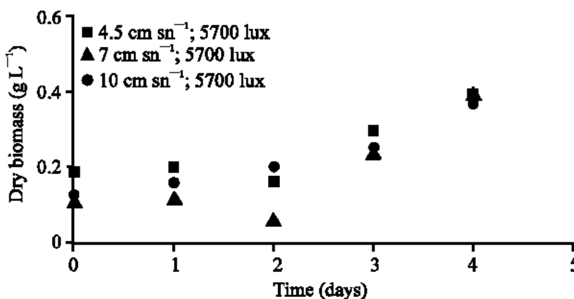

Fig. 5a: Variation of dry biomass (g L^{-1}) with different flow rates at 5700 lux

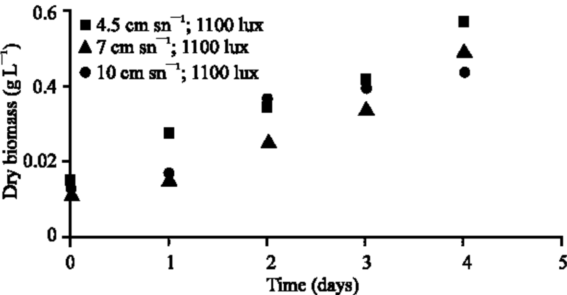

Fig. 5b: Variation of dry biomass (g L^{-1}) with different flow rates at 11000 lux

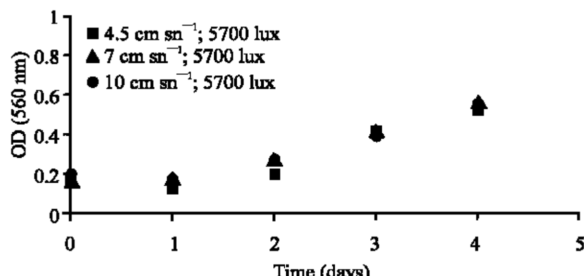

Fig. 6a: Variation of optical density with different flow rates at 5700 lux

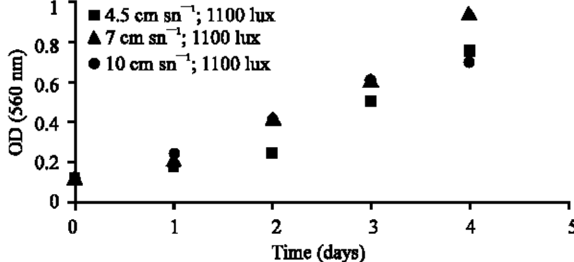

Fig. 6b: Variation of optical density with different flow rates at 1100 lux

Chlorophyll-a concentration reached a higher value of 48 mg L^{-1} with the sequential illumination mode. This could be an indication of the 12 h cycle effect in the coil unit that maintained a better illumination for the culture by

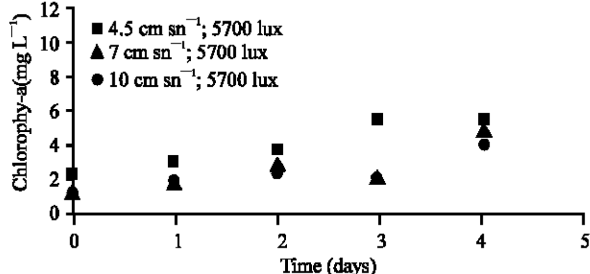

Fig. 7a: Variation of chlorophyll-a concentration with different flow rates at 5700 lux

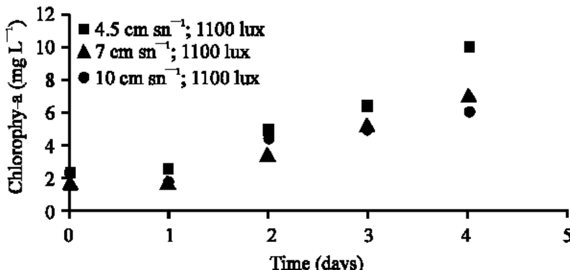

Fig. 7b: Variation of chlorophyll-a concentration with different flow rates at 11000 lux

taking consideration of the photosynthesis. These results agreement with other works like, Fox (1996) that found lower light intensity lead to higher chlorophyll-a concentration and lower biomass content for *Spirulina platensis* cultures.

After the 3 experiments to choose the best illumination mode the sequential mode was chosen. For the next experiments the different intensities were compared with different flow rates in the coil unit with sequential mode.

When pH levels were considered a similar change in both light intensities varied between the 8.8 and 9.8 interval. The different flow rates in the coil unit also did not effected slightly the pH levels (Fig. 4a,b). Dry biomass variation under 5700 lux light intensity made a change in the amount of dry weight. Maximum amounts of dry weight were nearly the same for the different flow rates (Fig. 5a). This was also similar to the results of the 11000 lux experiments but higher values reached. The flow rate of 4.5 cm sn^{-1} lead to an increase in both intensities. This was due to the longer time spend in the coil unit. Under high intensity the significance of the dry weight change was more effective compared to the low intensity with 4.5 cm sn^{-1} flow rate (Fig. 5b).

Optical density of the culture at 5700 lux did not show significant differences with various flow rates (Fig. 6a). When the light intensity was higher the optical density reached higher values compared to the lower light intensity (Fig. 6b).

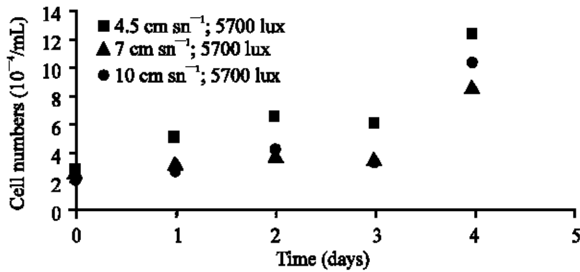

Fig. 8a: Cell number evolution with different flow rates at 5700 lux

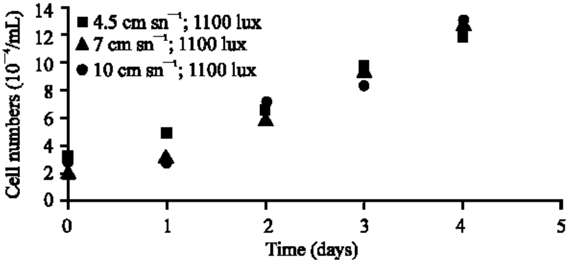

Fig. 8b: Cell number evolution with different flow rates at 11000 lux

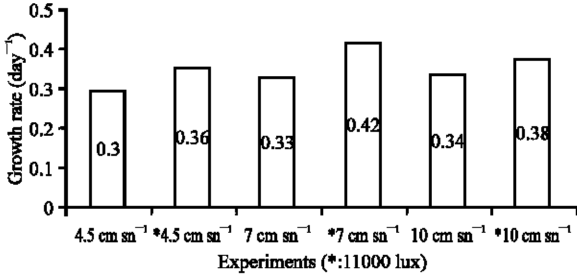

Fig. 9: Growth rate (day) comparison for different flow rates at two different light intensities

Chlorophyll-a concentration was not changed significantly for the different flow rates only a higher concentration was reached with a 4.5 cm sn^{-1} flow rate similar to the dry weight amount (Fig. 7a). Chlorophyll-a concentration was higher under high light intensity. The 4.5 cm sn^{-1} flow rate also caused a higher increase reaching 9.8 mg L^{-1} at 11000 lux (Fig. 7b).

Cell number changed similarly according to the light intensity in both cases. Cell number reached a higher value with 4.5 cm sn^{-1} at 5700 lux (Fig. 8a,b).

The experiments that focused on different flow rates were compared according to the growth rates reached. The highest growth rate was reached at 11000 lux with 7 cm sn^{-1} flow rate at the coil unit. The higher intensity maintained lead to a higher growth rate. Lower intensities were close at the growth rates (Fig. 9).

DISCUSSION

Production of *Spirulina platensis* in the integrated photobioreactor focused on the idea of photosynthesis. The simple formula of photosynthesis was basically the usage of water, light and CO_2 to produce energy for the organism. The design of the system was aiming to maintain a balance between two different units to have the best production. Two units were designed to complete each other. First unit was the main place of production which was a wide tank unit. At this unit the culture was illuminated continuously to maintain the energy for the culture in the wide tank. The tank unit was also the place of degassing where excess gas could be eliminated formed in the coil unit preventing photo-oxidation. In the coil unit the main idea was to have the best illumination for the photosynthesis and the place where the culture could be mixed through the coil tubes so the tank unit which was mixed pneumatically will not need an extra mechanical agitation system. The weakness of dark points in the wide tank unit was compensated with the continuous illumination with the internal lighting and also with the coil unit illumination. The problems like photolysis that can be caused by the narrow tubing in the coil unit tried to overcome by different illumination modes and flow rates with the intrinsic work with tank unit. The working harmony of two units were monitored with different parameters especially pH levels. The pH levels were tried to be stabilized in the system. The best results were obtained with the sequential illumination mode under 7 cm sn^{-1} flow rate. This was due to the balance between two units.

CONCLUSIONS

All the photobioreactor systems had different advantages and disadvantages and the idea of an integrated system was to unite the advantages of different systems. At this work the photobioreactor system was designed according to this idea. Another approach was to have a scale-up available system. This kind of PBR system can be utilized in a commercial type fermenter with simple modifications in the system. The internal illumination system can be employed in a fermenter easily. And the extra light could be supplied from an outdoor tubular system that would act as a support system to the main production unit. Even under the need of more light the artificial illumination could be employed in the coil system. In this design the tubular unit was employed as a support unit not the main production unit like in most of the designs focused on the tubular photobioreactors for production. So the scale up problems could be overcome especially about the high volume production with the tank units. And the land need for the tubular units by a helical vertical coil unit. For the future approach the integrated system was worth focusing on for heterotrophic, mixotrophic processes and for scale-up.

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