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Cultivation of Microalgae *Chlorella vulgaris* in Airlift photobioreactor for Biomass Production using commercial NPK Nutrients

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Abstract

Airlift reactors are widely used in the chemical and biochemical applications as effective contactors for mass and heat transfer. The main advantages of airlift contactor compared with simple bubble column are ease of construction, low shear rate, high capacity, good mixing and liquid circulation without mechanical agitators and circulating pumps.

In this work, growth characteristics of *Chlorella vulgaris* microalgae were studied in an internal loop airlift photobioreactor for biomass production. The bioreactor operated under batch and semi-continuous culture mode using commercially available 20:20:20+TE NPK fertilizer as nutrients. The experiments were conducted to evaluate the growth rate and biomass productivity of *Chlorella vulgaris* microalgae as affected by several factors such as nutrients concentration (20-80 mg/L), inlet air flow rate (2-8 LPM), and harvesting ratio (10-30 vol.%). The growth rate and biomass productivity of *Chlorella vulgaris* was determined as changes in optical density using UV-spectrophotometer. The results of batch operation showed that the growth rate of *Chlorella vulgaris* microalgae was increase with increasing of NPK nutrient concentration used but the access to the stationary phase of growth was delayed. The rate of growth was also increase with the increase in air flowrate to a limit then decrease. On the other hand the airlift photobioreactor can be operated in semi- continuous mode successfully by choosing the optimum conditions from the batch step which was 40 mg/L NPK nutrients concentration and 6 LPM and air flowrate. Several ratios of reactor content were harvested and the maximum biomass productivity was 0.142 g/L.day when harvested 10 vol.% every two days.

Keywords: *Airlift photobioreactor, Chlorella vulgaris, Microalgae, Biomass, NPK nutrients.*

1. Introduction

The production of biodiesel from algae biomass as a renewable source took a great interest in recent years. Algae biomass can produce oil more than the rest of biodiesel feedstocks such as soybean, corn, rapeseed, oil palm, and yellow grease per unit of growing area as well as algae can provide different types of renewable biomass such as carbohydrates, proteins and lipids [1].

Microalgae are microorganisms that can grow via photosynthesis process. It converts light, water and carbon dioxide into biomass typically found in freshwater and marine systems. Microalgae reproduction occurs primarily by vegetative asexual cell division [2].

In addition to the oil production, which can be used as biodiesel, there is a wide range of applications for microalgae products, from foods to wastewater treatment, aquiculture, and even hydrogen production [3].

The most productive microalgae studied by researchers for biomass production are Cyanophyceae (blue green algae), Chlorophyceae (green algae), Bacillariophyceae (diatoms) and Chrysophyceae (golden-brown algae). [1,3]

Chlorella vulgaris is one of the few microalgae can accumulate high content of lipids or carbohydrates under selected conditions. *Chlorella vulgaris* is a spherical single-celled green microalgae containing Chlorophyll-A and -B which it uses for photosynthesis. *Chlorella vulgaris* has potential in both foods other

industries such as pharmaceutical, cosmetics, and biofuel industries. *Chlorella vulgaris* is known as one of the fastest growing green microalgae. The optimum conditions for cultivation of *Chlorella vulgaris* are temperature of 20 - 25 ^{o}C and pH of 4 – 7 [3, 4].

Chlorella vulgaris biomass when dried consists of, approximately, 40% protein, 25% oil, 20% carbohydrate, 5% fiber, and 10% minerals and vitamins. Despite its highly efficient photosynthesis which allows it to produce more oil and protein than most other plants. The lipid content in *Chlorella vulgaris* could be increased significantly between 50% and 70% [5].

Airlift reactors are contacting devices with two interconnecting and distinct zones only one of which is usually sparged by gas called riser whereas the other region is called downcomer which does not receive the gas. The different gas holdup in the gassed and ungassed zones results in different bulk densities of the fluid in these regions which causes circulation of the fluid in the reactor by a gas-lift action. Generally airlift contactors exist in two forms internal loop and external loop. In internal loop reactor, regions are separated either by a draft tube or a split cylinder while in external loop, riser and downcomer is separated physically by two different tubes. Mixing is done by bubbling the gas through sparger in the riser tube without any physical agitation. Riser is similar to bubble column where sparged gas moves upward randomly and haphazardly [6].

Because the simplicity in design and construction, clear flow patterns, good mixing, low required mechanical power, less shear for a given quality of mixing, and many other advantages airlift reactors may be used for several industrial applications whether for two or three phase systems including aerobic fermentation (such as citric acid, biomass from bacteria and vinegar), microorganism cultivation, and metal bearing wastewater treatment [7].

As a comparison between the four main types of photobioreactors used for microalgae production, table (1) show the typical advantages and disadvantages of these closed systems [8].

Table 1,
Typical advantages and disadvantages of the four main types of closed bioreactors for microalgae cultivation.

Reactor type	Typical advantages	Typical disadvantages
Fat plate reactor	• shortest oxygen path • low power consumption	• low photosynthetic efficiency • shear damage from aeration
Tubular/ horizontal reactor	• high volumetric biomass density	• oxygen accumulation photoinhibition • most land use
Airlift/Vertical reactor	• best exposure to light/dark cycles • greatest gas exchange • least land use • high photosynthetic efficiency • high liquid circulation	• support costs • scalability
Bubble column/Vertical reactor	• best exposure to light/dark cycles • greatest gas exchange	• Low photosynthetic efficiency
CSTR	• good mixing • Easy to scalable	• high power consumption

2. The Objective

The objective of the present work is to cultivate the *Chlorella vulgaris* microalgae in internal loop draught tube airlift photobioreactor for biomass production using commercial 20:20:20+TE NPK fertilizer as low cost nutrients under different experimental conditions. The experimental conditions studied in this work include nutrients concentration (20, 40, 60, and 80 mg/L), inlet air flowrate (2, 4, 6, and 8 LPM), harvesting ratio (10, 20, and 30 vol.%) and operation mode (batch and semi-continuous mode).

3. Experimental

3.1. Sample of Microalgae Strain

Collection and purification of *Chlorella vulgaris* strain was done at the Market Research and Consumer Protection Center, University of Baghdad using serial dilution culture method.

After receiving the isolated *Chlorella vulgaris* strain the sample was immediately incubated in the algal-culture room under controlled conditions of temperature (20-27°C), photoperiod (18 hr light/4 hr dark) and light (1 k lux by two florescent tubes each 13 watt). These cultures were maintained in five 0.5 L flasks (300 mL working volume), with aeration with filtered air and using 20:20:20+TE NPK fertilizer with concentration of 30 mg/l as nutrients (composition, preparation and sterilization of culturing medium are showed in the later sections). The cultures were subcultured every 8 days by transferring cells to a fresh medium (10 ml culture was added to each flask and then completed to 300 ml working volume of fresh media solution). Figure (1) shows the starter culture of *Chlorella vulgaris* microalgae (in flasks) while Figure (2) shows the Photographic View of the batch cultivation using N:P:K+TE fertilizer media with time in day.

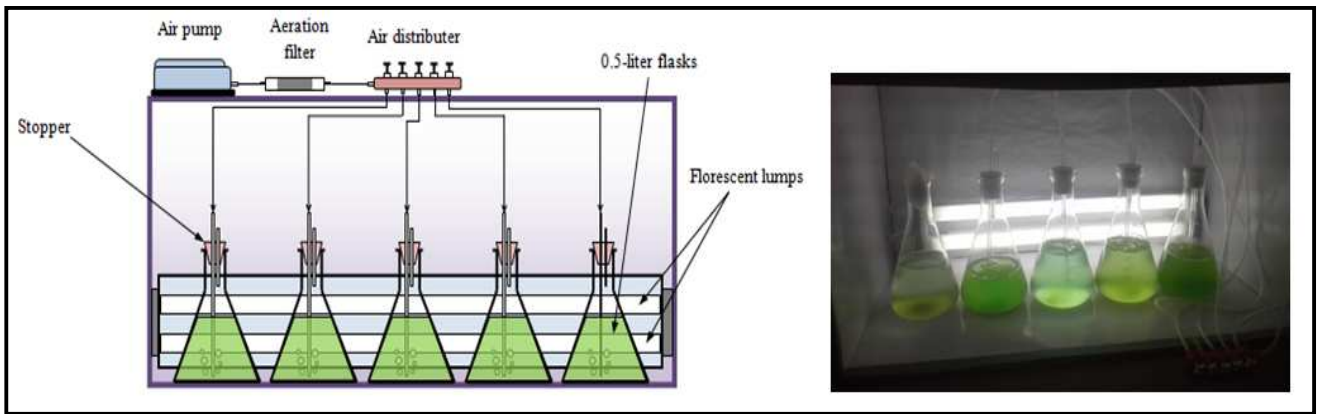


Fig.1. Experimental setup of *Chlorella vulgaris* strain incubate

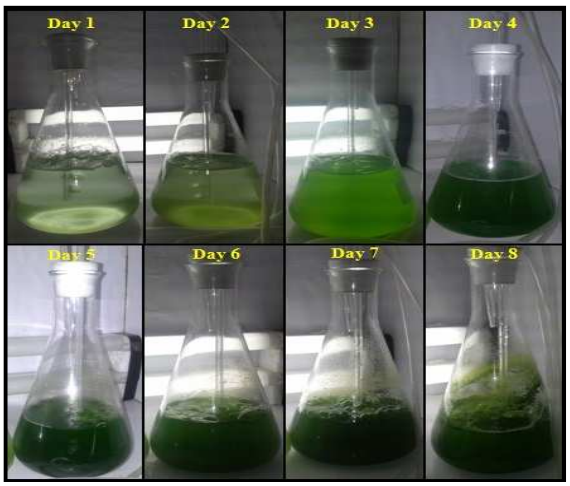


Fig. 2. Photographic view of the *Chlorella vulgaris* microalgae cultures with time.

3.2. Preparation of Culturing Medium

Commercial available N:P:K 20:20:20+TE fertilizer (provided from Kule® Inc.) was used as high water soluble and low cost nutrients for biomass culture with combination of 20:20:20+TE. Table (2) shows the composition of each element in this commercial NPK fertilizer. Four concentration of NPK fertilizer 20, 40, 60, and 80 mg/L were used in this work by dissolving the appropriate amount of the NPK fertilizer in RO water.

Table 2,
Composition of commercial N:P:K+TE fertilizer.

Constituents N:P:K 20:20:20	**Concentration % (Nitrogen:Phosphorus:Potassium)**	
N as urea	2.1%	Total **N** = 20%
N as ammonia	17.9%	
P as phosphorus oxide	20%	
K as potassium oxide	20%	
Mg	0.1 %	
Zn	0.05 %	
Mn	0.05 %	
Fe	0.1 %	
Cu	0.05 %	
B	0.02 %	
Vitamin B	0.0005 %	

3.3. Equipment and Procedure

Before conducting cultivation experiments in the bioreactor and to minimize the risk of contamination or infection by potential pathogens and other microorganism, species in the flasks and medium the nutrient media, glasses and equipment must to be sterilized. The simple and alternative method used for sterilization of nutrients solution was by using a gas stove as the arrangement shown in Figure (3) where a flask with nutrient medium solutions are kept in the hot water at a temperature of (70-80°C) for about 20-30 minutes. The nutrient medium in the flask should not be allowed to boil as this will vary the nutritional value of the medium.

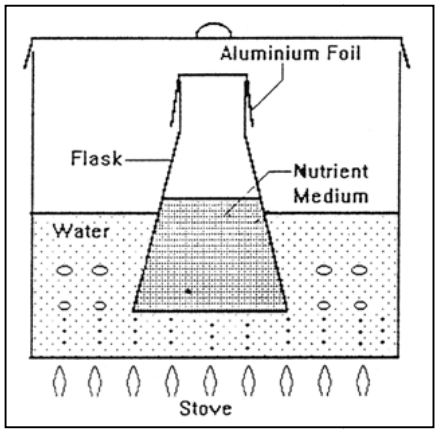


Fig. 3. A way of sterilization of flasks and nutrients medium.

To avoid contamination by condensation in the air tube airlines, the air should be dried by filtration through an in-line filter before entering the riser section of the airlift photobioreactor; therefore aeration filter unit contain cotton and activated charcoal must be used as shown in Figure (4).

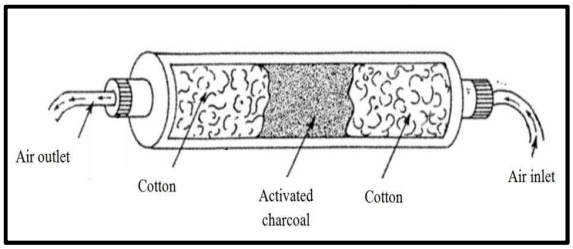
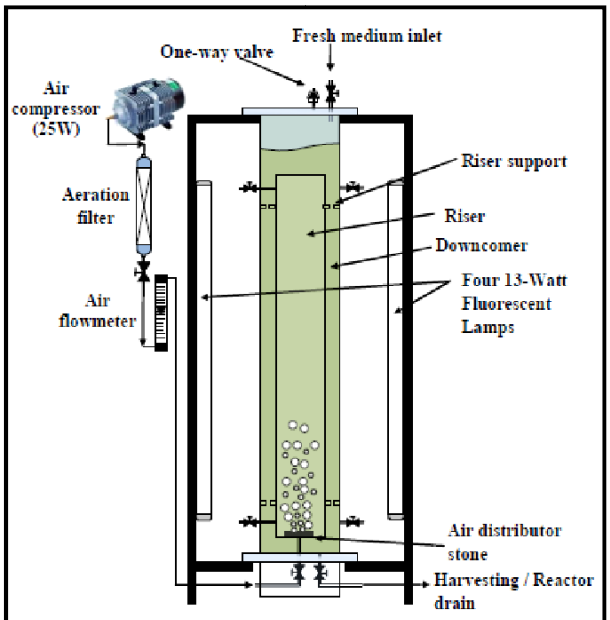


Fig. 4. Aeration filter used in avoid contamination in the air lines.

Also the airlift photobioreactor was sterilised by fuelled with a solution of about 200 ppm Sodium metabisulfide ($Na_2S_2O_5$) as a decontamination agent hold in the airlift bioreactor for about 20 minute.

The airlift photobioreactor used in this work, shown schematically in Figure (5), consisted of two concentric columns made from transparent Perspex material. The internal tube (which is the riser) is 8 cm diameter and 60 cm height while the outer tube is 80 cm height and 12 cm diameter. The air is passed through the internal column to become the riser this was done by 25W, 2 bar pressure air compressor. The air distribution was done by small air porous stone. Four fluorescent tubes (13 watt each) were used to provide the light to the bioreactor. The height of internal tube (riser) from the bottom is 5 cm that give a free area about 251 cm^2. The photobioreactor is sealed so that no air enters from the outside to the inside this was done by using one-way valve. Figure (6) show a photographic view of the experimental airlift photobioreactor in use.



Dimension	Value (cm)
Downcomer diameter (D_d)	12
Riser diameter (D_r)	8
(D_d/D_r)	1.5
Bottom clearance L_b	5
Top Clearance	≈5
Internal tube (Riser) height (H_r)	60
External tube height (H_d)	80
Reactor working volume (liter)	6.6

Fig. 5. Laboratory airlift photobioreactor schematic.

Fig. 6. photographic view of the laboratory airlift photobioreactor in use.

The experimental procedure was departed in two stages batch and semi-continuous mode. In all experiments the culture conditions of cultivation temperature, light and dark photoperiod cycle and pH were kept constant at 20-25 ^{o}C, 20:4 hr, and 4 – 7 respectively.

- **Batch operation stage**

The purpose of this stage is to access to the highest level of growth rate of microalgae *Chlorella vulgaris* and the best conditions of NPK nutrients concentration and air flowrate were recorded to be used in the next stage (semi-continuous operation). Therefore two sets of experiments were performed. In the first set four levels of NPK nutrients concentration were used (20, 40, 60, and 80 mg/L) and fixing air flowrate on 2 LPM while in the second set the air flowrate were varied into four values (2, 4, 6, and 8 LPM) and the NPK nutrients concentration was fixed on the value which gives a higher growth rate and shortest generation time from the first set.

In all experiments 250 ml of stock culture was used then complete the reactor to its working capacity (6.6 liter) with RO water containing prespecified NPK nutrients concentration. For counting about 1 ml sample was removed every day from the bioreactor and kept in a stoppered tube with 1 drop of iodine, which killed the cells.

Microalgae concentration was determined indirectly by optical density than by direct counting of cells or by cell dry weight. The optical density (cell absorption) was measured using UV- Visible spectrophotometer by measuring absorbance at a wave length of 540 nm [9].

Calibration curve for relationship between optical density and cell dry weight was formed by the following procedure [10]:

The sample of microalgae was firstly centrifuged at 3000 r/min for 20 min to settling it into the bottom then it was dried by exposure to atmosphere for 24 hr and then dried at 105°C overnight. After drying, the algae were powdered. Five culture samples were tested and the equation which is relating the biomass concentration in g/L with the optical density (cell absorption) in nm was determined. The equation is given in results and discussion section.

The growth rate (K) and generation time (G) were also can be calculated by the following equations as given by [12]:

$$K = \frac{\ln\left(\frac{X_t}{X_0}\right)}{t_2 - t_1} \quad \ldots(1)$$

$$G = \frac{\ln 2}{K} \quad \ldots(2)$$

Where X_t and X_o= final and initial populations at time t_2 and time t_1, respectively. Because the culture was monitored every day therefore t_2-t_1=1.

- **Semi-continuous operation stage**

This stage started after selecting reactor conditions air flowrate and NPK nutrients medium concentration which give a best growth rate curve and after reaching the stationary phase of microalgae growth rate, a part of bioreactor content were removed by a ratio of 10, 20 , and 30 % as a harvesting ratio. Then the bioreactor content compensated with a fresh medium all at once at the same time (i.e. fresh medium with prespecified concentration flows into the bioreactor, and the spent culture with prespecified harvesting ratio flows out) then the culture is allowed to grow for a given period and then the procedure is repeated for another harvesting ratio.

In general Table (3) shows the experimental setup and conditions for cultivation of *Chlorella vulgaris* microalgae in airlift photobioreactor.

Table 3,
Experimental conditions for biomass culture of microalgae in airlift photobioreactor.

Parameter	Value
Cultivation temperature	20-25 ^{o}C
pH	4-7
Alternate light and dark photoperiod cycle	20:4 hr
Air flowrate (LPM)	2, 4, 6, and 8
NPK nutrients concentration (mg/L)	20, 40, 60, and 80
Liquid work volume in the bioreactor	6.6 liter
Harvesting ratio	10, 20, and 30 %

4. Results and Discussion

4.1. Batch Mode Runs

The initial period of the airlift bioreactor operation was in a batch mode using different concentration of NPK nutrients and air flowrate as described in experimental section. The growth curves of *Chlorella vulgaris* microalgae in term of cell absorbance (nm) at different NPK nutrients concentration and at air flowrate of 2 LPM were illustrated in Figure (7).

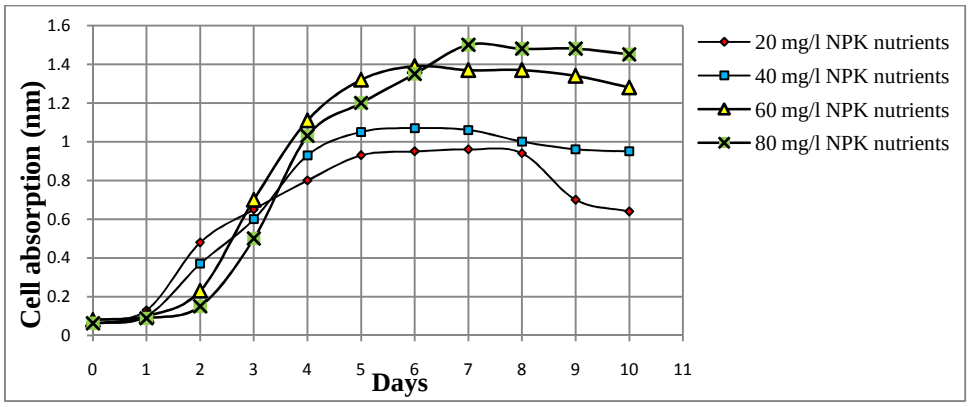


Fig. 7. Growth curves of Chlorella vulgaris microalgae at different nutrients concentration and at air flowrate 2 LPM.

All the growth curves showed about four growth phases of microalgae cultures. The first phase is induction or lag phase which little increase in growth rate as cell absorption (nm) occurs. In the second phase the growth rate increases exponentially this is depends on many factors such as algal species or type, light intensity and medium temperature. The third phase is the constant phase in which the cell density become relatively constant. Finally declining growth rate when the cell divisions decreases because some factors become influential to growth rate such factors as NPK nutrients concentration, pH, dissolved CO_2, light and contamination risk.

Since *Chlorella vulgaris* is a heterotrophic and autotrophic, it can be easily cultivated in various synthetic media and even in sewage [11]. So the 20:20:20+TE NPK nutrients often succeed in culturing *Chlorella vulgaris* microalgae and at a reasonable rate.

At levels of 20 and 40 mg/L NPK nutrients concentration the growth of *Chlorella vulgaris* entered the stability phase in 5 days while at 60 and 80 mg/L NPK nutrients concentration entered the stability phase in day 6 and 7 respectively.

Since nitrogen is the main limiting nutrient to microalgae growth [12], very high concentration in the media of microalgae growth may cause deactivation in the production of pigments necessary for photosynthesis [13]. Therefore the high levels of NPK nutrients concentration (60 and 80 mg/L) needs longer time to reach to the stability phase of growth.

The calibrated data between the optical density and cell dry weight is shown in Figure (8).

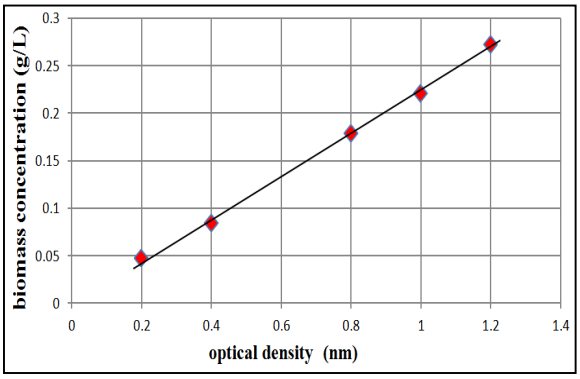


Fig. 8. Calibration curve for relationship between optical density and cell dry weight.

The five tested culture samples which are used to create the equation relating the biomass concentration in g/L with optical density (cell absorption) in nm by plotting these data and the equation is:

$$Y=0.211\ X \qquad \ldots(3)$$

Where the value Y is the biomass concentration (g/L) and the value X is the optical density (cell absorption, nm).

The *Chlorella vulgaris* microalgae cultivation in the batch mode showed similarities in growth

behavior in all four levels of the NPK nutrients concentration used. The final biomass dry weights were 0.317 g/L in 80 mg/L nutrients concentration medium, 0.298 g/L in the in 60 mg/L nutrients concentration, 0.225 g/L in 40 mg/L nutrients concentration and 0.2 g/L in 20 mg/L nutrients concentration.

The maximum growth rate (K) and generation time (G) at each level of nutrient concentration can be calculated and shown in Figures (9) and (10) respectively. It is clear that the maximum value of growth rate (1.30834 g/L.day) and shortest generation time (0.5298 day) where reported for 40 mg/L nutrient concentration.

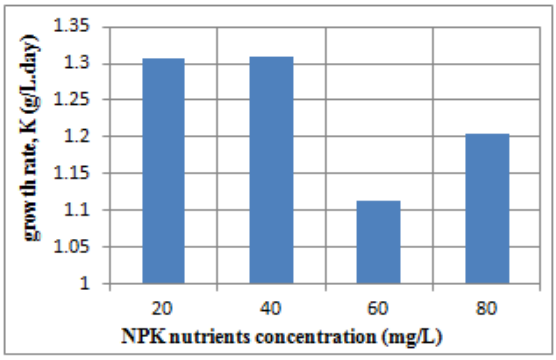


Fig. 9. Growth rate of Chlorella vulgaris microalgae at different nutrients concentration.

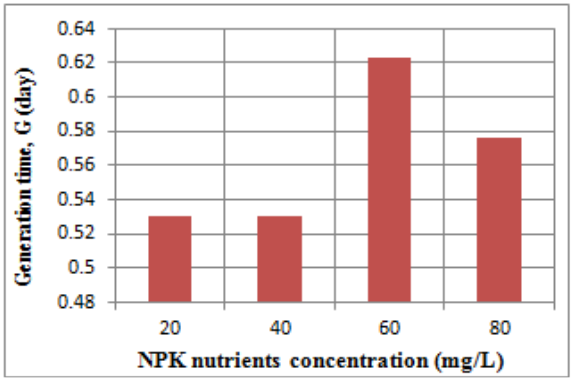


Fig. 10. Generation time of Chlorella vulgaris microalgae at different nutrients concentration.

Therefore the nutrient concentration of 40 mg/L was selected to examine the effect of air flowrate in airlift photobioreactor. Figure (11) shows the effect of air flowrate on growth of *Chlorella vulgaris* at 40 mg/L NPK nutrient concentration.

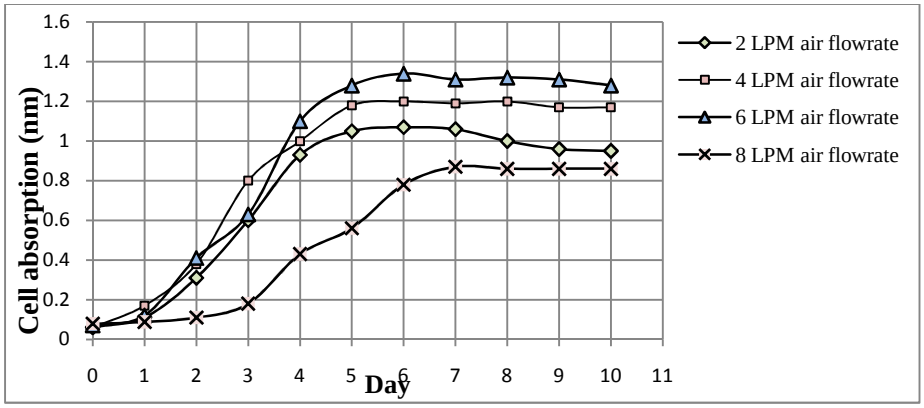


Fig. 11. Growth curves of Chlorella vulgaris microalgae at different air flowrate and at 40 mg/L NPK nutrient concentration.

As shown in the Figure (11) the cell absorption increase with increasing air flowrate up to 6 LPM then decrease at 8 LPM. Maximum growth rate and shortest generation time were achieved at about 6 LPM as shown in Figure (12). This result can be explained when increasing air flowrate the holdup in the reactor increase and the mass transfer rate increase therefore the rate of growth of microalgae enhanced. But when further increasing in air flowrate above 6 LPM this increased gas holdup and bubble size decrease, the column becomes hazy leads to do not penetrate the light into the depth of the column and therefore decreases the growth rate. This is also explained by [14, 15].

The best conditions in batch runs were reported as 6 LPM air flowrate and 40 mg/L NPK nutrient concentration. These conditions were used in the experiments of semi-continuous mode runs.

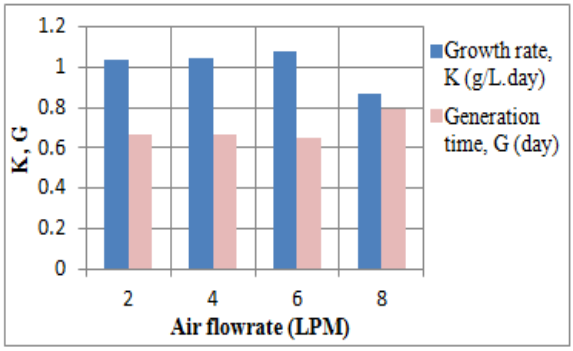


Fig. 12. Growth rate and Generation time at different air flowrate

4.2. Semi-Continuous Mode Runs

Three different harvesting ratios (sometimes referred to as the dilution rate) were examined 10, 20, and 30 vol.%. The air flowrate and NPK nutrient concentration were kept constant at 6 LPM and 40 mg/L respectively. The culture was continued for 28 day and the results are shown in Figure (13).

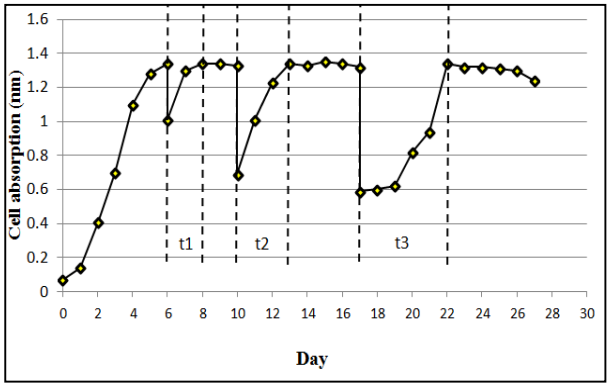


Fig. 13. Grown behavior of Chlorella vulgaris microalgae under *semi-continuous mode conditions*.

It can be seen from Figure (13) when 10 % vol. was harvested from the bioreactor, the system takes two days (t_1) to return to the stationary phase, three days (t_2) for 20 % vol. harvesting and five days (t_3) for 30 % vol. harvesting.

Steady-state conditions were achieved when a constant up-and-down pattern in the cell absorption was observed for each harvesting ratio. At the highest harvesting ratio (30 %vol.), when the cell absorption stabilized (steady-state condition), the up-and-down cell absorption ranged from 1.34 nm to 1.01 nm. At the middle harvesting ratio (20 %vol.), the cell absorption ranged from 1.33 nm to 0.69 nm. At the lowest harvesting ratio (10 %vol.), the cell absorption ranged from 1.32 nm to 0.59 nm.

Figure (14) shows the effect of harvesting ratio on the biomass productivity in g/L.day. It is clear that the maximum biomass productivity was 0.142 g/L.day every 2 days.

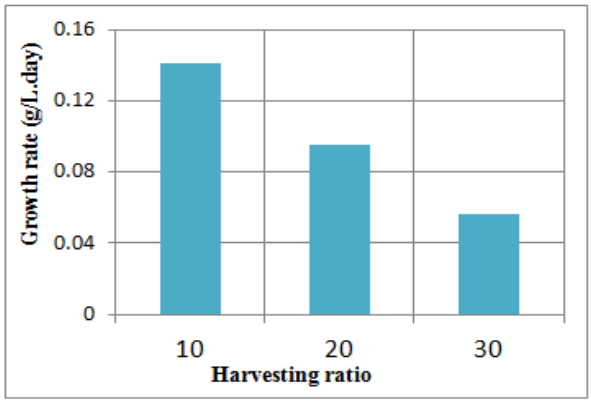


Fig. 14. Biomass productivity (growth rate) at different harvesting ratio.

5. Conclusions

Biomass production can be successfully accomplished by *Chlorella vulgaris* microalgae cultivation in an experimental Concentric-draught tube airlift photobioreactor designed and constructed in the laboratories of chemical engineering department, Al-Nahrain University. The photobioreactor operated under two modes batch and semi-continuous mode and using very low cost and available commercial 20:20:20+TE NPK nutrients. Any required biomass productivity of *Chlorella vulgaris* microalgae can be obtained and ranged from 0.0565 g/L.day at 30 vol.% dilution ratio to 0.142 g/L.day at 10 vol.% dilution ratio.

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استنبات الطحالب الدقيقة *Chlorella vulgaris* في مفاعل الرفع الهوائي الحيوي الضوئي لانتاج الكتلة الحيوية بأستخدام سماد NPK التجاري

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الخلاصة

في هذا العمل، تم دراسة خصائص النمو للطحالب الدقيقة *Chlorella vulgaris* في مفاعل الرفع الهوائي الحيوي الضوئي ذي التدويرالداخلي لإنتاج الكتلة الحيوية. المفاعل الحيوي المستخدم يعمل تحت نظامين هما النظام الدفعي والنظام شبه المستمر وباستخدام مغذيات واطئة الكلفة ومتاحة تجاريا وهي سماد الـ NPK ٢٠:٢٠:٢٠+TE. أجريت التجارب لتقويم معدل النمو و إنتاجية الكتلة الحيوية للطحالب وتأثرهما بعدة عوامل مثل تركيز المغذيات (٢٠-٨٠ ملغ / لتر)، ومعدل تدفق الهواء الداخل (٢-٨ لتر / دقيقة)، ونسبة الحصاد (١٠ -٣٠ ٪ حجما).

وأظهرت النتائج العملية للنظام الدفعي أن معدل نمو الطحالب الدقيقة *Chlorella vulgaris* يزداد مع زيادة تركيز المغذيات المستخدمة ولكن الوصول إلى مرحلة ثابتة من النمو يتأخر كذلك يزداد معدل النمو بزيادة معدل جريان الهواء في المفاعل الحيوي الى حد معين بعدها ينخفض. من ناحية أخرى يمكن تشغيل مفاعل الرفع الهوائي الحيوي الضوئي في وضع شبه المستمر بنجاح وذلك باختيار وتطبيق افضل الضروف المستحصلة من عملية النظام الدفعي من تركيز المغذيات و معدل جريان الهواء والتي كانت ٤٠ ملغم / لتر و ٦ لتر/ دقيقة على التوالي تم حصاد عدة نسب من محتوى المفاعل وان أقصى قدر من انتاجية الكتلة الحيوية المستحصلة كانت ٠.١٤٢ (غرام / لتر.يوم) عند نسبة حصاد ١٠ ٪ حجما كل يومين.