Cultivation of *Chlorella Vulgaris* Using Airlift Photobioreactor Sparged with 5%CO_2-Air as a Biofixing Process

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ABSTRACT

The present paper addresses cultivation of *Chlorella vulgaris* microalgae using airlift photobioreactor that sparged with 5% CO_2/air. The experimental data were compared with that obtained from bioreactor aerated with air and unsparged bioreactor. The results showed that the concentration of biomass is 0.36 g l^{-1} in sparged bioreactor with CO_2/air, while, the concentration of biomass reached to 0.069 g l^{-1} in the unsparged bioreactor. They showed also that aerated bioreactor with CO_2/air gives more biomass production even the bioreactor was aerated with air. This study proved that application of sparging system for cultivation of *Chlorella vulgaris* microalgae using either CO_2/air mixture or air has a significant growth rate, since the bioreactors become more thermodynamically favorable and provide impetus for a higher level of production.

Key words: microalgae, *chlorella vulgaris*, airlift photobioreactor, CO_2 biofixing process

زراعة الكلوريلا فيلغارس في مفاعل الايرلفت مع ضخ خليط 5 CO_2% -هواء كعملية تثبيت بايلوجية

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الخلاصة

البحث الحالي يتناول دراسة زراعة طحالب الكلوريلا فيلغارس باستخدام مفاعل حيوي ضوئي الايرليفت بتهوية خليط من 5٪ CO_2 / الهواء. وتم مقارنة البيانات التجريبية مع التي تم الحصول عليها من مفاعل حيوي ضخ مع الهواء النقي فقط ومفاعل حيوي اخر بدون تهويه. النتائج أظهرت أن تركيز الكتلة الحيوية هو 0.36 g l^{-1} في مفاعل حيوي مع خليط من CO_2 / الهواء، في حين، تركيز الكتلة الحيوية وصلت إلى 0.069 g l^{-1} في مفاعل حيوي بدون تهوية. هذه النتائج اثبتت أن مفاعل حيوي التي ضخ اليه خليط من CO_2 / الهواء يعطي مزيدا من إنتاج الكتلة الحيوية من الزراعة في مفاعل حيوي ضخ اليه الهواء فقط. أثبتت هذه الدراسة أن تطبيق نظام التهوية لتفعيل نمو طحالب الكلوريلا فيلغارس باستخدام خليط من CO_2 / الهواء أو الهواء النقي فقط يعطي معدل نمو كبير، لأن المفاعلات الحيوية تصبح أكثر ملاءمة للديناميكية الحرارية وتوفر حافزا لمستوى أعلى من الإنتاج.

الكلمات الرئيسية: الطحالب، كلوريلا فيلغارس، مفاعل حيوي ضوئي الايرلفت، عملية تثبيت CO_2 بايلوجيا.

1. INTRODUCTION

Until recently, fossil fuel as a main source of energy is considered. However, concern about the depletion of this source is still a big challenge for the researchers. Therefore, the finding convincing alternatives to meet this challenge is necessary. Fossil fuels are also one of the reasons changing the climate by increasing the greenhouse gases (GHGs) such as carbon dioxide, methane, water vapor, nitrogen oxide and sulphur oxide **Amaro et al., 2011; Demirbas, 2011; Lee et al., 2010; Ranjan et al., 2010, Liu, 1994**. Among these gases, carbon dioxide is the most plentiful of gas and represents about 68% of the estimated total (GHG) emission **Harrington and Foster, 1999; Kondili and Kaldellis, 2007; Roman, et al., 2007.** In 1997, more than 170 countries signed the Kyoto agreement to reduce carbon dioxide emissions **Gutierrez et al., 2008.**

In addition, many studies have concerned biological process such as CO_2 mitigation from environment by fixing mechanism **De Morais and Costa, 2007a.** Some studies have used the terrestrial plants as source of biodiesel fuel for example; palm and soybeans **Costa and De Morais, 2011; Demirbas and Demirbas, 2011.** This alternative requires a long time to be ready for use and requires a large areas of farmland that definitely affect the production of food crops and increasing the global food crisis **Ozkurt, 2009; Demirbas 2010 ; Kecebas et al,2009; Demirbas 2011 ; Costa and de Morais, 2011; Demirbas and Demirbas, 2011.** Therefore, with these challenges, the researchers used another biological process which is less expensive and more economic through using the microalgae for CO_2 mitigation and convert it into biomass production **Carvalho et al., 2011**. In general, cultivation of microalgae requires sunlight as a source of energy, nutrients, water, and carbon dioxide as a source of carbon and regulation of pH in the medium **Chisti, 2007; Carvalho et al., 2011.** The microalgae have other advantages over terrestrial plants crops including, higher growth and biomass production rates, and shorter maturity rates. Normally microalgae double their biomass within 24 hours. Also, it required far less land for growing thus which is not compromising food production and supply, and oil content reaches to 20-50% in its biomass **Chisti, 2007; Clarens et al., 2010; Lee et al., 2010; Mercer and Armenta, 2011**. The cultivation of microalgae is obtained in a closed engineering system such as airlift reactor, which has recently gained renewed interest as a promising strategy for carbon dioxide mitigation **Filali et al, 2011.** This system has a great potential productivity due to better control of the environment and harvesting efficiency. Furthermore it has optimized space/volume utilization, therefore, more efficient use of costly land **Pedroni et al., 2001.**

Furthermore, supplying the carbon dioxide represents one of the major limiting factor in scale up the bioreactor design **Richmond, 2008; Morita et al., 2000**. Therefore, using CO_2 as sparging gas in the microalgae cultivation helps to strip oxygen accumulated and prevent toxicity of algae cells **Ying et al., 2013.** The current research represents an objective study which has been carried out to verify the effect of the proportion of carbon dioxide with the air on the growth of *Chlorella vulgaris*

microalgae by using airlift photobioreactor that was designed by different working group **Al-Mashhadani et al., 2015.**

2. MATERIAL AND METHOD

2.1 Microalgae Culture and Cultivation Medium

Microalgae strain used in the current experiments is *Chlorella vulgaris*, which was isolated and purified at Plant Laboratory for Graduate Studies, Department of Biology, College of Science, University of Baghdad by using serial dilution and different plating techniques as described by **Abed et al, 2014**. This type of microalgae was collected from three different places in drinking water treatment plant that is located on Tigris River and purified by serial dilution. The stock cultures were propagated and maintained on NPK medium (20:20:20+TE N: P: K) commercial fertilizer. Its advantages are its being low-cost nutrients and high water soluble for biomass culture **Ammar, 2016.** This fertilizer has the following composition as percent concentration (N as urea 2.1% and as ammonia 17.9% , P as phosphorus oxide 20% , K as potassium oxide 20% with trace element consist of Mg 0.1% , Zn 0.05% , Mn 0.05% , Fe 0.1% , Cu 0.05% , B 0.02% and Vitamin B 0.0005%). This medium was prepared by dissolving these salts in RO treated water, the pH (6.23) was adjusted by using (0.1 N) of NaOH and HCl.

The incubation conditions used were continuously illuminated by three cool-white fluorescent light tubes (T5 Led, china), each fluorescent tube was 10 Watt and 2 foot length mounted approximately 10 inches at the surface of the flasks. Agitation was carried out by bubbling continuously sterile and (2.3) filtered air into the stock culture to provide a constant source of CO_2. The weekly subculture of algae cells was made to maintain the inoculum.

2.2 Design of Lab Scale Airlift Loop Photobioreactor

Fig.1 shows the configuration of airlift bioreactor used in the present study. The design of the airlift bioreactor was made according to results obtained from **Al-Mashhadani et al., 2015** using Navier-Stokes equations as main equations in their modeling. However; some modifications have been done to get a suitable environment of microalgae culture. The modified bioreactor was fabricated out of Plexiglass pipe, with dimensions of 350 mm in height and 150 mm in diameter. The airlift loop bioreactor consists of a stone diffuser located in the center of concentric tubes with 80 mm in diameter, fixed at distance 30 mm from the bottom and internal draught tube with dimension 100mm in diameter, 250 mm in height and fixed at 50 mm above the bottom of the reactor, 6 mm above the stone diffuser . Holes were made on the top of the reactor for fixing the props and exhaust gas.

2.3 Experiment Setup and Culture Condition

Fig. 2 illustrates the setup of photobioreactors for *Chlorella vulgaris* cultivation with lab scale airlift loop reactor. Both reactors have the same dimensions and were provided with magnetic stirrers (MRHEI-Tec, Germany) to ensure that all cells are exposed to the light and to prevent microalgae sedimentation. The microalgae cells were continuously illuminated over 24h per day by three fluorescent cool white lights, while the temperature of the culture media was controlled by aquarium heater controller (HK 50, China) at 30°C.

PH meter and dissolved oxygen (DO) props were fixed in downcomer region for daily measurement. The bioreactors were aerated with air that was mixed with 5% CO_2 from a gas cylinder for 28 minutes every day during the cultivation time. The CO_2/air mixture was adjusted in small mixture provided with a gas analyzer (Biogas 5000, G Geotech, UK, England) to achieve a desired concentration of CO_2 in the airstream and the flow rate was regulated by the gas flow meter at 200ml/min. The sparging gas mixture was filtered and sterile through inline air filtered 20 mm in diameter which was inserted in the bottom of the reactor as can be seen in **Fig. 2**. The air was supplied by air pump (HX-106A, China) and mixed with CO_2 when it is required. The second rector culture was operated with no additional CO_2, except that already existed in the air supplied.

The pre-cultured of *Chlorella vulgaris* was inoculated into the reactors with an inoculum size 250ml that was fed to the reactor and completed with 5L of NPK, as working volume and operating in a batch mode under 30°C. During this time, the airlift photobioreactor was sterilized by filled with a solution of about 200 ppm of odium Meta bisulfide (Na_2SO_4) as a decontamination agent hold in the airlift bioreactor for about 20 minutes before inoculation.

In this work, two sets of experiments were conducted. In the first experiment, one of the airlift bioreactor cultures was dosed with 5% CO_2 enriched with air. The dosing was periodically for about 28 min per day at a rate 200 ml /min, the dosing was carried out until pH reached 6. While the second bioreactor was unaerated (control) for comparison purpose with the cultures aerated.

The second experiment was conducted to represent a comparison between one aerated with mixture of air and 5% CO_2 and another aerated with only air at the same aeration time. After gas dosing, 5ml of samples of each culture was taken to evaluate kinetic parameter. PH and dissolved oxygen were measured before and after dosing.

2.4 Analytical Determination

Chlorella cultivation in the airlift reactors was tested for up 11days and during this time, sampling process was carried out every 24h intervals. Microalgae growth rate was estimated by two different methods. The first method measures the optical density (OD) at wavelength 680 nm (i.e., OD_{680}). This wavelength was chosen on preliminary tests for estimation the maximum absorbance (optical density) at wide range of wavelength. Researchers used similar wavelength **Mohd et al., 2011** and

Jeong et al., 2003 by using UV spectrophotometer (GENESYS 10UV, USA) to ensure maintaining an exponential phase of growth. The second method was measuring the dry weight of microalgae (g l^{-1}) by filtering the samples after centrifuged it in (Centrifuge PLC- 03, Taiwan) with 3000 r/min for 20 min then dried at 60 ^{0}C for 1h. A linear regression equation was derived to describe the relationship between biomass dry weight (g l^{-1}) and corresponding OD at 680 nm wavelengths.
The pH value for each culture was measured daily before and after gas dosing using pH meter (CRISON, Basic 20, USA). The dissolved oxygen (DO) level of the cultures was also measured before and after gas using (CRISON, OXI 45+, Spin).

3. KINETIC PARAMETER

Growth curve of density with time was accomplished by the biomass concentration (X, g l^{-1}) that can be used to estimate maximum specific growth rates (μ, d^{-1}), biomass doubling time (td, d), maximum biomass concentration (X_{max}, g l^{-1}) and volumetric biomass productivities (P, g l^{-1} d^{-1}) for each cultures.
Specific growth rate, μ (day^{-1}) was estimated from Eq.(1) **Chiu et al., 2009.**

$$\mu = \frac{Ln \frac{X_t}{X_o}}{\Delta t} \quad (1)$$

Where X_t and X_0 are dry biomass concentrations (g l^{-1}) respectively during the exponential logarithmic growth phase, and Δt is the cultivation time in the day during the exponential logarithmic growth phase **Ketheesan and Nirmalakhandan, 2012.**

Doubling time (td, d) was calculated from Eq. (2)

$$t_d = \frac{Ln\, 2}{\mu} \quad (2)$$

Biomass productivity, P (dry g l^{-1} day^{-1}) in batch mode was estimated from the variation in biomass concentration within the cultivation time (day) according to Eq. (3) **Ryu et al, 2009.**

$$P = \frac{(X_t - X_o)}{t} \quad (3)$$

Where X_t is the dry biomass concentration (g l^{-1}) at t (day) and X_0 is the dry biomass concentrations at inoculation **De Morais and costa, 2007a**.

According to the method described by **Ketheesan and Nirmalakhandan, 2012,** the carbon dioxide biofixation rate F_{CO2}, (g_{CO2} day^{-1}) can be estimated from Eq. (4)

$$F_{CO2} = a\ P\ V_C \quad (4)$$

Where P is the biomass productivity of the culture (g l^{-1} d^{-1}), V_C is the culture volume in liter and (a) is the mass of CO_2 fixed in unit biomass which calculated by Eq. (5)

$$a = Cc\ (M_{CO2}/\ M_C) \quad (5)$$

Where Cc is the carbon content of the dried microalgae biomass (g carbon (g biomass)$^{-1}$), since 50% of carbon content in the dry biomass **Becker, 1994**, M_{CO2} is the molecular weight of CO_2 and M_C is the molecular weight of Carbon.

4. RESULTS AND DISCUSSION

Chlorella vulgaris growth was investigated in airlift bioreactor to determine the photosynthetic efficiency in this reactor. According to the suggested experimental design, two experiments were carried out to estimate the aeration effect on *Chlorella* growth at constant temperature and illumination.

Effect of aeration on growth of microalgae

In the first experiment, *Chlorella* was cultivated in batch airlift bioreactors. The sparging system was carried out using ambient air enriched with 5% pure CO_2 and the results were compared with no aeration culture (unsparged bioreactor). **Fig.4** shows the growth curves for *Chlorella vulgaris* cultivation. From this figure, it can be seen that the algae cells have a close biomass concentration during the first day (lag phase) in reactor sparging with CO_2. After adaption, cells began good growth during the experiment culture. Whilst, cells cultivated in control reactor took a long period for adoption. The maximum biomass concentration was obtained from a culture aerated with 5% CO_2. The cell concentration recorded about 0.36 (g l^{-1}) after 11 days. While, the growth rate with absence of aeration (control) has no significant increase, and maximum concentration of the cells was about 0.069 (g l^{-1}) at 10^{th} day then began inhibited.

In the second experiment, *Chlorella* was also cultivated in batch airlift bioreactors. But, one of the bioreactors was sparged with ambient air enriched with 5% pure CO_2, while the other bioreactor was sparged with ambient air only for the same period of sparging (28 min) in both reactors.

Fig.5 shows the growth curve of *Chlorella* cells aerated with air enriched with 5% CO_2. It has the same behaviour of that in the first experiment except the adaption period, although the experiments were carried out at a different time. This gives a kind of credibility the results of those experiments.

However, in the first experiment culture, the microalgae cells require one day for adaption, while in the second experiment, the response displayed no adoption (lag phase). This behaviour was because the stock cultures already were maintained under 5% CO_2. **Schimidell et al., 2001** demonstrated that when the inoculums culture are maintained under different conditions from those used in the cultivation runs, the adaptation (lag) phase probably will be long. But it absents when the conditions are similar. **Yun et al., 1997** also checked that acclimatization of the inoculum is important and found that when stock cultures of *Chlorella vulgaris* were maintained under air, it grew less well in media containing 15% (v/v) additional CO_2, but when the inoculum was grown under air supplemented with 5% CO_2, it grew better in culture media containing 15% additional CO_2 than it did in media containing 5% CO_2.

As can be seen from the **Fig. 5**, *Chlorella* cells also reached to the 0.359 g l^{-1} as maximum biomass concentration in 11 day under aeration with 5% CO_2, which is approximately the same result obtained in the first experiment. While the growth rate without any addition of CO_2 only atmospheric air, the growth rate was slow. The microalgae biomass concentration reached to 0.056 g l^{-1} at 3rd day and remained stable at this value for few days then increased slowly to reach maximum concentration (X_{max}) 0.083 g l^{-1} at 11th day to be double concentration compared with its inoculum. The important kinetic parameter for *Chlorella* cultivate in both experiment are summarized in **Table (1).**

The experimental data showed higher maximum specific growth rate, maximum biomass concentration, maximum biomass productivity and minimum doubling time under culture with CO_2 enrichment with 5% CO_2 for both experiments. The highest value of specific growth rate was (0.39 day^{-1}) while the lowest value of specific growth rate was (0.17 day^{-1}) for *Chlorella* species cultivated with 0.036% of CO_2 in ambient air. Whereas, no significant value in specific growth for control because of the very slow growth and unobtainable exponential phase. In addition, the shortest biomass doubling time (td, day) was 1.7 day at 5% CO_2 aerated, while the longest td was 3.9 days at ambient air aerated as a notice in **Table 1.** Therefore, as maximum growth rate increased, biomass doubling time decreases and cultivation becomes more economically sustainable. So microalgae can duplicate their biomass in less than 7 days, whereas higher plants take many months or years **Vonshak et al., 1982**. In this study, the doubling time was equal to or less than 4 days.

The productivity (g l^{-1} per day) of *Chlorella* species cultivated under 5% CO_2 for both experiments reached to the maximum value of 0.031 (g l^{-1} per day), while 0.0077 (g l^{-1} per day) was obtained for culture aerated with air only. The 0.0036 (g l^{-1} per day) which is the maximum value was obtained per day for control.

Fig.6 shows the photographic view of algae cultivation in the two experiments. It can be seen that there is significant difference between the cultures using high concentration of CO_2 and the ambient air or control in the present study.

The results in this study are approximately similar to other studies that cultivated *Chlorella* species in photobioreactor under aeration of 5% CO_2, for example; **Chiu et al., 2008** found when *Chlorella* sp. cultivate under 5% CO_2 with continuous aeration and illumination the specific growth rate was (μ_{max}= 0.34 day^{-1}) and cell dry weight was (X_{max}= 0.899 g l^{-1}) for high density inoculum about ($8x10^6$cells ml^{-1}). While, he obtained (X_{max}= 0.062 g l^{-1}) as cell dry weight and (μ_{max}= 0.127 day^{-1}) for low-density inoculum about ($8x10^5$cells ml^{-1}). **De Morais and Costa, 2007a, b** found that the best kinetics of *Chlorella* is (μ_{max}= 0.31 day^{-1}) and doubling time is (td=2.27 day). The maximum productivity was (P_{max}= 0.14 g l^{-1} per day) for *Chlorella* cultivated under 6% CO_2 for 15 min in every hour during the illumination with 12 hr light / 12 hr dark photoperiod.

Effect of aeration on carbon dioxide biofixation

Dissolved carbon dioxide and fixation by microalgae through the photobioreactor was detected during each experiment. From the previous studies it depended on the law of material conservation. It allows for calculating carbon dioxide biofixation rate from estimation the microalgae biomass production rate (g l^{-1} d^{-1}). In which, the carbon content of the biomass (g_{CO2} /g biomass) approximately ranges from (0.5 - 0.57). Some of calculations were conducted using a report biomass molecular formula ($CO_{0.48}$ $H_{1.83}N_{0.11}P_{0.01}$) **Chisti, 2007.** These calculations were used when direct biofixation rate of CO_2 was not available, which is based on the assumption that biofixation rate of CO_2 in the form of extracellular products was negligible.

In this study, CO_2 biofixation rate was determined according to the method described in **Becker, 1994** that is similar to use by other researchers i.e., **Ho et al., 2010 and Tang et al., 2011.** It was found that the fixation rate of CO_2 resulted in high value when CO_2 concentration increased in air. As shown in **Table 1.**, the maximum biofixation rate of CO_2 was (0.28 g_{CO2}/day) observed at 5% CO_2 when 5L culture sparged with 28 min per day at a rate of 200 ml/min. While it was (0.069 g_{CO2}/day) when the ambient air was contained approximately 0.036% of CO_2.

A good similarity was noticed between the values obtained from this study and the values from the following researchers; **Tang et al., 2011** found that CO_2 fixation rate was (0.244 g_{CO2}/day) for *Chlorella* cultivated in 1L Erlenmeyer flask when continuously aerated with 5% CO_2 enriched air. Whereas, he found the fixation rate was (0.134 g_{CO2}/day) for culture continuously aerated with 0.036% CO_2., **Sydney et al., 2010** found CO_2 fixation rate was (0.251 g_{CO2} /day) for *Chlorella vulgaris* cultivated in 8 L working volume continuously aerated with 5% CO_2.

Some researchers had other results of CO_2 fixation rate for *Chlorella* cultivated such as; **Ryu et at., 2009** who found CO_2 fixation rate was (0.35 g_{CO2}/day) for *Chlorella* species cultivated with 5% CO_2 concentration in 600 ml bioreactor under continues aeration, **Lv et al., 2010** recorded CO_2 fixation rate (0.6 - 1.2) at maximum value in 5L photobioreactor aerated with hollow fiber membrane sparging with 0.5% CO_2; And **Scragg et al., 2002** who found the biofixation of CO_2 for *Chlorella*

aerated at ambient air at 25 ^{0}C was (0.04 g l^{-1} per day) and (0.024 g l^{-1} per day) in Wayamba's media and Low N medium respectively.

Photosynthetic activity of the culture medium containing *Chlorella* cells was determined by pH and dissolved oxygen (DO) monitoring. Both of measurements were carried out daily before and after dosing of air enriched with 5% CO_2 or with air only (without bubbling).

Effect of aeration on pH of the culture

As can be seen in **Fig. 7** and **Fig. 8**, the behaviour of culture curves aerated with 5% CO_2 are the same. The periodical supplement of CO_2 to the culture resulted in almost steady pH level in the target region (6.18 – 9.02) without the use of expensive buffer and the periodic dosing made pH controlled in the expected range. So this results in a potential for both energy and economic saving. While for the culture aerated by air only a gradual increase in PH was noticed and this increased caused algae inhabitation as noticed in **Fig. 8**. Commonly, as microalgae growth, the pH in the culture increased due to cellular metabolism which converted CO_2 uptake through photosynthesis to the carbonic acid (H_2CO_3), then the last was dissociated into bicarbonate (HCO_3^{-1}) of the medium. CO_2 is uptake by microalgae metabolism at a rate depending on the activity of the photosynthesis, which itself depended on the light activity. This causes increasing in associate to H^+ ion and increasing in pH, but as a consequence of pH increasing (CO_3^{-2}) increased also. When HCO_3^{-1} and CO_2 decreased causes photosynthetic reaction inhabitation and the approximately of the algae trichomes and (OH^-) ion are formed making pH become more alkaline which improves the rate of algae respiration **Steenman, 1975; Fox, 1996.** Therefore the many algae cultures need to use buffer or acid added when pH of culture increased over the suitable level via an auto-controlled culture.

In this study neither acid nor buffer was added because it was expected that the increasing pH could be neutralized by daily CO_2 supply by dosing technique. While noticing the pH of control culture also increased very slowly continually until the 10th day with its dry weight increased from 0.03g l^{-1} to only 0.06 g l^{-1} as a record in **Fig. 4** .Corresponding , its pH also increased slowly and rising from (7 - 8.32) as shown in **Fig. 7**. Then, when pH values not changes, this means the algae began died. The culture aerated only air also grew slowly to reaches a maximum value at 9th day with its dry weight increased from (0.03 g l^{-1} to 0.074 g l^{-1}) as seen in **Fig. 4**. Correspondingly, its pH increased slowly from (7- 9.2) as shown in **Fig. 8**. However, after 9 days the pH barely increased and was maintained at (9.21 - 9.25). Whilst the algae almost stopped growing as well, with the cell dry weight (0.07 - 0.083 g l^{-1}) because of absence CO_2 supplied and O_2 accumulation in the culture can cause adverse effects on the growth of alga. The reasons are that; after pH went above 9 value, the culture of microalgae was inhibited because for most type of microalgae the suitable pH ranges from 6 to 9 **Coutteau, 1996.** And over this ranges inhabitation in growth occurs.

In the culture aerated by air enrichment with 5% CO_2, the cell dry weight increased dramatically from a range (0.03 – 0.36) g l^{-1} during the experiment culture, the corresponding pH was assumed to

rise even faster than the culture aerated with air only or the control culture. However, due to dosing daily so the pH of the culture maintained in a suitable range. As can be seen from **Fig. 7** and **Fig. 8**, the pH values from a range (7.89 - 9.02) as heights value before dosing to the range (6.16 - 6.18) as low value after 28 min of CO_2 dosing daily. Because of the desirable culture condition (CO_2 unlimited and O_2 free), the pH value reduced to this range then returned to increase back to about (7.89 - 8.34) within only one day ago. The next day, another 28 min of dosing dragged it back to around the range (6.16 - 6.18) again. Such a virtuous cycle kept pH within a desirable range, making the culture also not limited by pH. Therefore these results indicate that the increasing CO_2 concentration led to improving algae biomass productivity.

Effect of aeration on DO of the culture

Dissolved oxygen (DO) also was determined through the experiments culture in airlift photobioreactor. It is a point to photosynthesis activity. During the lighting process, microalgae metabolism occurs as a result of photosynthetic generation, in which the oxygen concentration increases steadily. While carbon dioxide disbursed by microalgae and reached to saturation state with culture medium, this causes dissolving level dropped steadily and DO rejected through metabolism. In addition, the initial O_2 value was generated in the culture through which air bubbled.

Fig. 9 and **Fig. 10** show the daily values of DO measurement for both experiments. As noticed from **Fig. 9** there is no change for control culture curve and the DO stay stable through the run. This refers to low photosynthesis activity as a result of no aeration occurs. While in another culture when air aerated which made mixing in the reactor and decreases in the DO value. Through the culture runs, air enriched with 5% CO_2 dosing periodically at 28 min per day found decreases of DO in a suitable range from (20.1 – 22.9 mg l^{-1}) as highest value before dosing to (8.24 – 8. 9 mg l^{-1}) as low value after dosing daily. This process kept the DO in the culture safety. Increasing the microalgae biomass through the culture aerated with 5% CO_2, resulted of consequently increased accumulation of the DO in culture, as shown in **Fig 9** and **Fig 10**. Whilst, the reactor culture when aerated with air only, DO values range from (12.2 -15.6 mg l^{-1}) as highest value before dosing to (8.35 – 8.76 mg l^{-1}) as low value after dosing daily. Whereas, DO of the control culture recorded as increased from (12.4 to 13.9 mg l^{-1}) during the cultivation experiment. This slowly increased occur due to the low growth of the cells without aeration. In addition, the mixing of culture efficiently by bubbling air is very important for microalgae cultivation in a closed reactor, because the high elevated dissolved oxygen concentration level can lead to severe photo-oxidation which damage microalgae cells and causes decreasing the treatment efficiency **Oswald, 1988; Lee and Lee, 2003; Richmond, 1991.** For instance, oxygen level above air saturation (0.2247 mole O_2 m^{-3}) could inhibit photosynthesis in many microalgae species, even if carbon dioxide concentration is maintained at elevated levels **Aiba, 1982.** Generally, the mixing not only reduces the DO in microalgae cultures but also ensures good mass transfer of carbon dioxide and oxygen in the culture system **Ugwu et al., 2007.**

5. CONCLUSIONS

The present article studied the behavior of *Chlorella vulgaris* microalgae in airlift photobioreactor. The growth rate and biomass production was experimentally achieved with *Chlorella vulgaris* culture in airlift photobioreactor. The airlift bioreactor as draft tube proved promising technology for obtained optimal mixing. Moreover, the designed system has successfully shown ability to sequester CO_2 from airstream containing 5% CO_2 concentration. As well, the *Chlorella vulgaris* has very effective to quantitatively remove CO_2 from an elevated CO_2 airstream in laboratory bioreactor. The main results showed higher growth rate and biomass production can be achieved if the sparging system was 5% CO_2/Air compare with air only. For example, maximum growth rate was (0.39 d^{-1}) and maximum cell concentration was (0.36 g l^{-1}). While with sparging air, the maximum growth is (0.17 d^{-1}) with (0.038 g l^{-1}) cell concentration.

In addition, this study showed the high CO_2 biofixation potential by *Chlorella vulgaris* microalgae was 0.28 g_{CO2} per day. The periodic dosing strategy of CO_2 is proposed for *Chlorella* cultivation as well. Daily 28 minutes of 5% CO_2 enriched with airstream supplement to the culture maintained pH at a suitable level (6.18 - 8.3) without using any expensive buffer for adjustment the pH value. Removal of oxygen from the culture was investigated in the current study. The aeration system decreases the DO concentration of the culture in a suitable range from (22 mg l^{-1} to 8.29 mg l^{-1}) after periodically dosing daily.

6. ACKNOWLEDGEMENT

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8. NOMENCLATURE

a	mass of CO_2 fixed in unit biomass
Cc	carbon content of the dried microalgae biomass, (g carbon (g biomass)$^{-1}$)
d	day
F_{CO2}	carbon dioxide biofixation rate, (g_{CO2} day^{-1})
M_{CO2}	molecular weight of CO_2, (g gmol^{-1})
M_C	molecular weight of Carbon, (g gmol^{-1})

P	biomass productivity, (g l^{-1} d^{-1})
P_{max}	maximum volumetric productivity, (g $l^{-1}d^{-1}$)
t	time, (day)
td	doubling time, (day)
V_C	culture volume, (L)
X_t	biomass concentration at any time t, (g l^{-1})
X_0	biomass concentration at the inoculation, (g l^{-1})
X_{max}	maximum biomass concentration, (g l^{-1})
μ_{max}	maximum specific growth rate, (day^{-1})
Δt	the cultivation time in day during the exponential growth phase

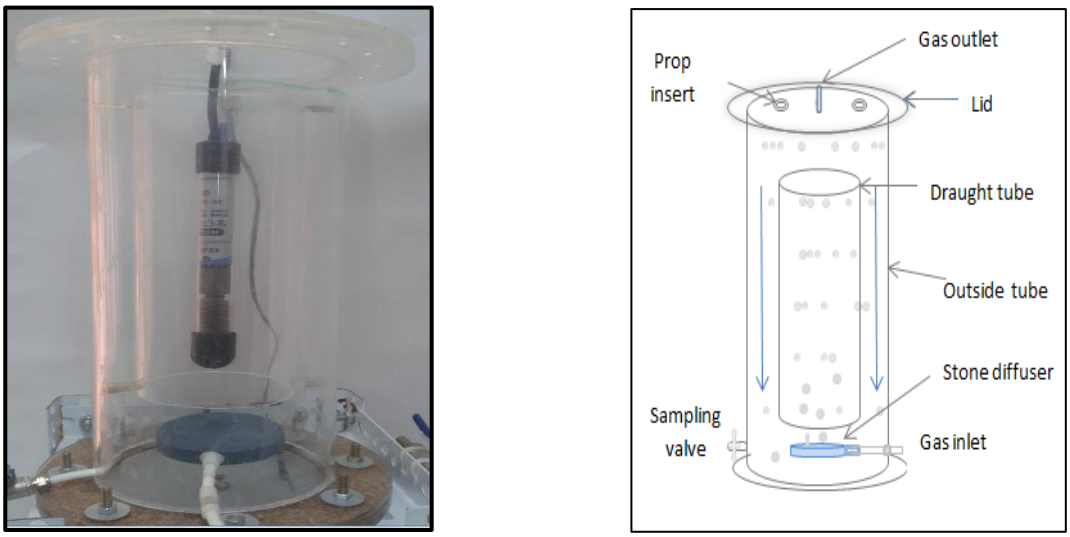

Figure 1. The structure of airlift loop bioreactor**.**

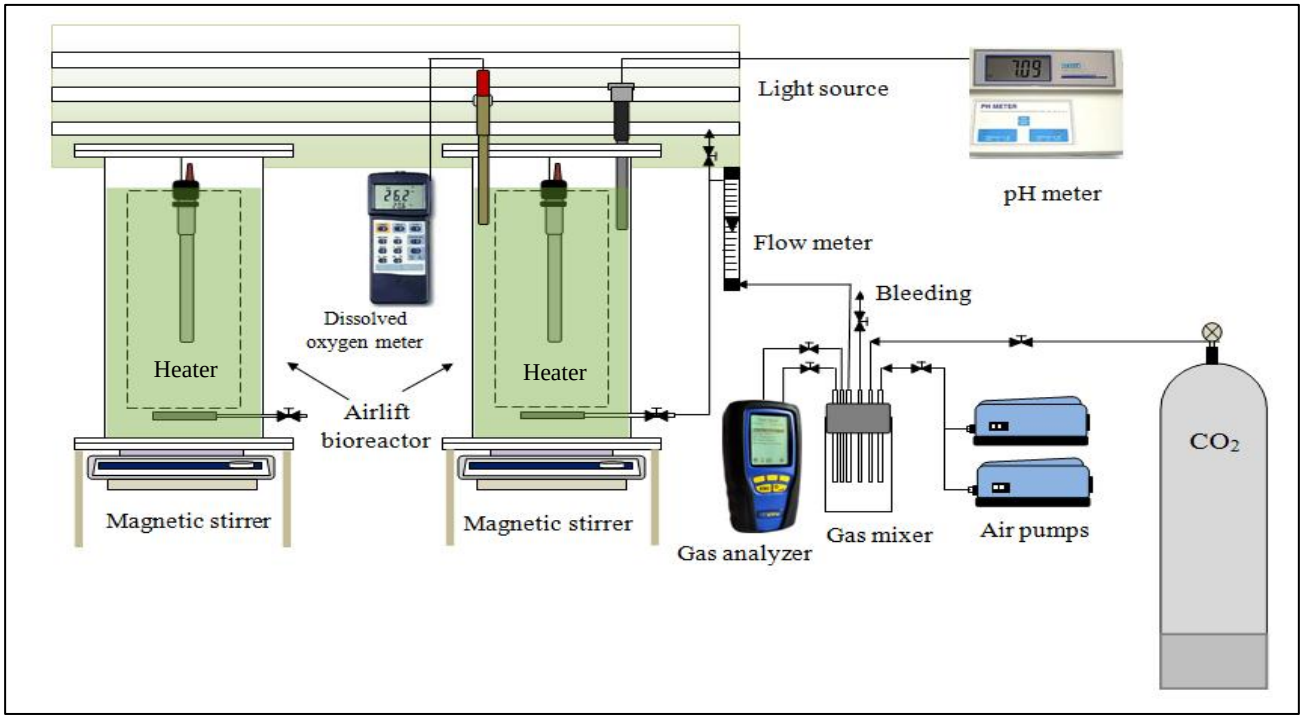

Figure 2. Schematic diagram of the photobioreactor setup.

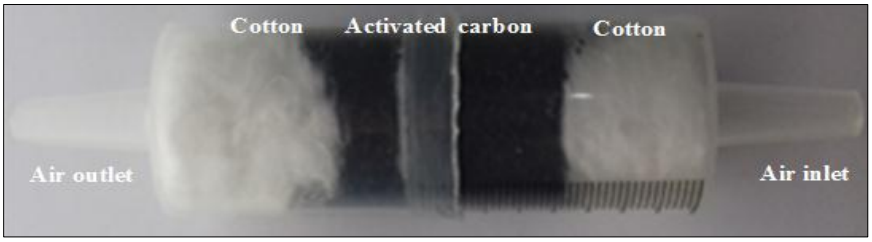

Figure 3. Aeration filter used in avoiding contamination in the airlines.

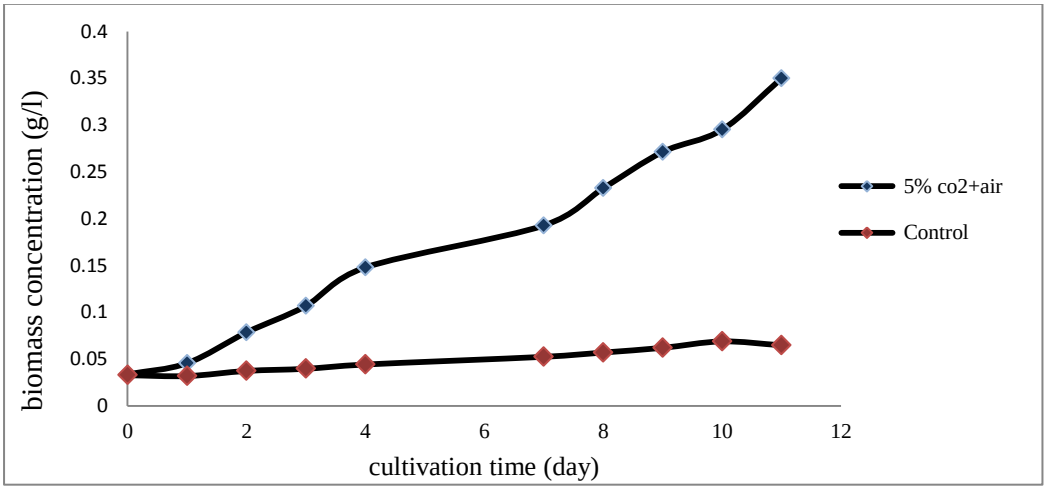

Figure 4. Effect of the concentration of CO_2 sparging on the growth of *Chlorella vulgaris*. The culture was inoculated with 0.033 gl^{-1} of microalgae cell approximately and cultivate without aeration (control), air enriched with 5% CO_2 bubbled at 200 ml/min for 28 min per day under continues illumination and 30 ^{0}C temperature.

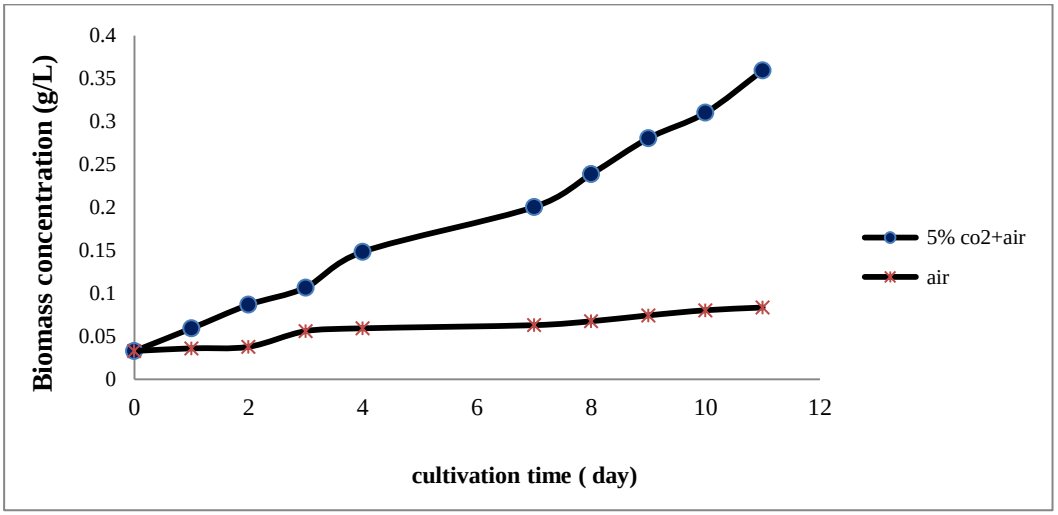

Figure 5. Effect of the concentration of CO_2 aeration on the growth of *Chlorella vulgaris* .In the culture approximate 0.033g l^{-1} of microalgae cell was inoculated and cultivate under atmospheric air, air enriched 5% CO_2 bubbled at 200 ml/min for 28 min per day under continues illumination and 30 ^{0}C temperature.

Table1. Maximum specific growth rate (μmax, d^{-1}), doubling time (td. d), Maximum biomass concentration (g l^{-1}/d) and CO_2 fixation rate (FCO2, g_{CO2} /day) for *Chlorella vulgaris* in airlift photobioreactor.

	CO_2 aeration	μ_{max} (day^{-1})	td(day)	X_{max} (gl^{-1})	P_{max}($gl^{-1}d^{-1}$)	F_{CO2}(gco_2 per day)
Experiment(1)	Control	-	-	0.069	0.0036	-
	Air with5%CO_2	0.39	1.76	0.36	0.031	0.28
Experiment (2)	Air	0.17	3.9	0.083	0.0076	0.07
	Air with5%CO_2	0.39	1.7	0.359	0.031	0.28

(-) means no significant value obtained.

(a) (b)

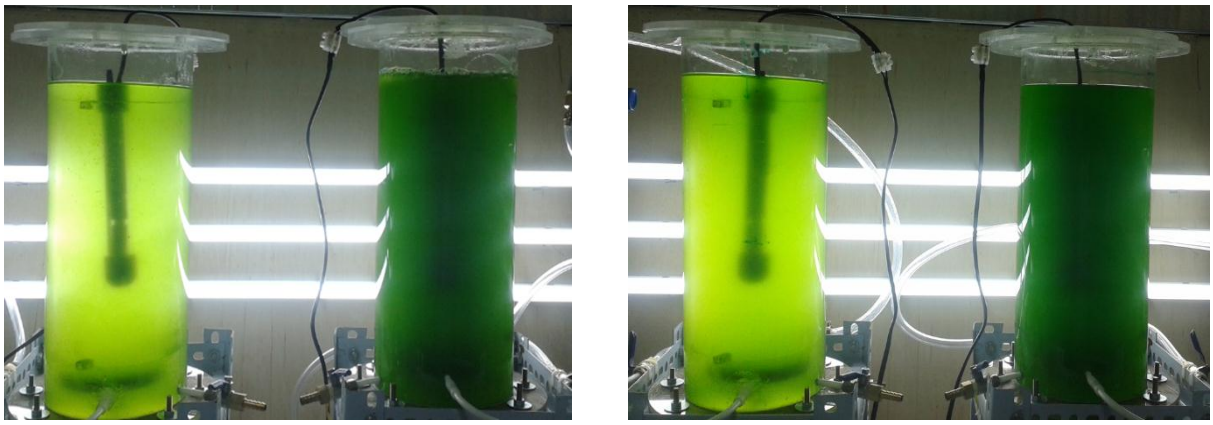

Figure 6. Photograph of cultivation *Chlorella vulgaris* in the two experiments (a) Comparision between cultivation under air enriched with 5% CO_2 and control (no aeration). (b) Exp.2. Comparision between cultivation under air enriched 5% CO_2 and ambient air only.

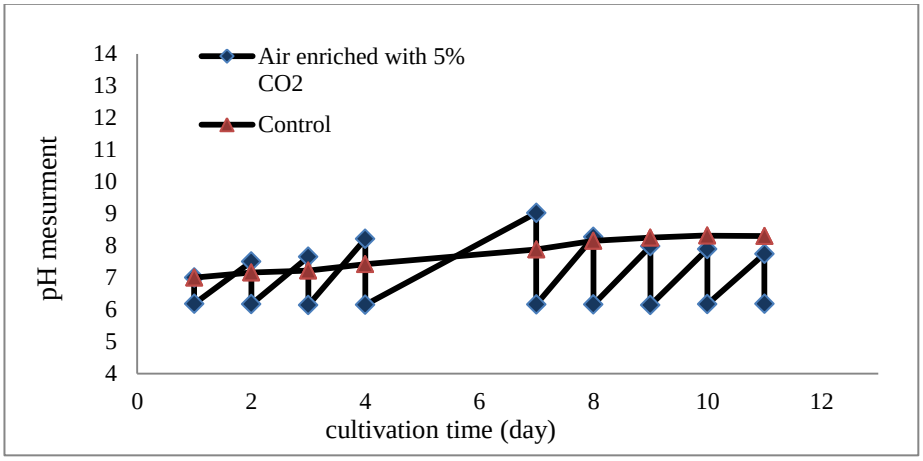

Figure 7. Daily pH values changes with culture time for *Chlorella vulgaris* supplied periodically 5% CO_2 for 28 min per day compare with control. There are two pH values per day, a higher value one and a lower value, representing the pH values before and after dosing, respectively.

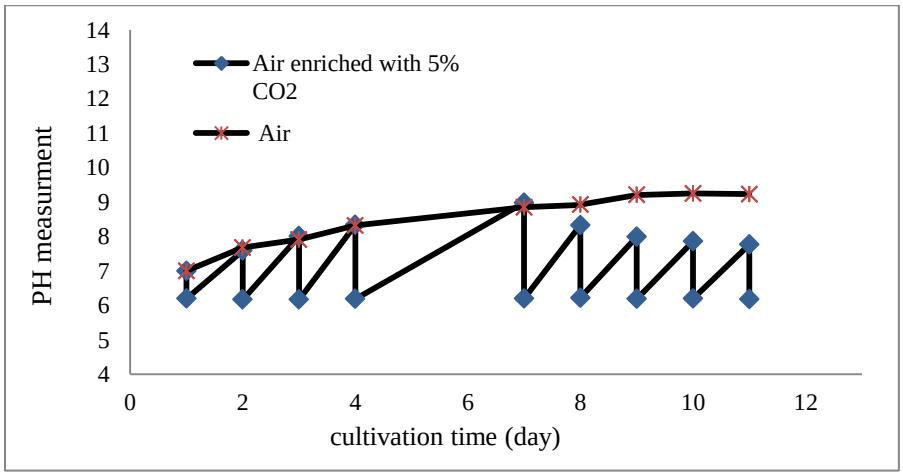

Figure 8. Daily pH values changes with culture time for *Chlorella vulgaris* supplied periodically 5% CO_2, air for 28 min per day. There are two pH values per day, a higher value one and a lower value one, representing the pH values before and after dosing, respectively.

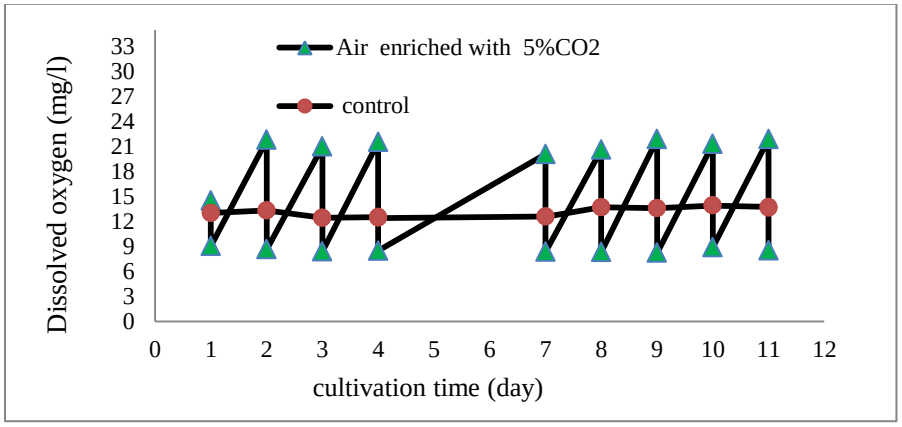

Figure 9. Daily DO values changes with culture time for *Chlorella vulgaris* supplied periodically 5% CO_2 for 28 min per day compare with control. There are two DO values per day, a higher value one and a lower value one, representing the DO values before and after dosing, respectively.

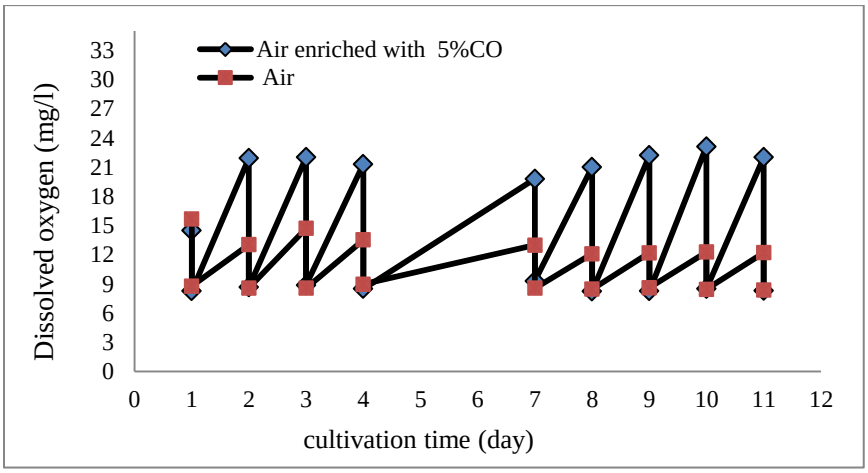

Figure 10. Daily DO values changes with culture time for *Chlorella vulgaris* supplied periodically 5%CO_2, air for 28min per day. There are two DO values per day, a higher value one and a lower value one, representing the DO values before and after dosing, respectively.