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Experimental Study for Commercial Fertilizer NPK (20:20:20+TE N: P: K) in Microalgae Cultivation at Different Aeration Periods

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Abstract

Recently, microalgae have become a promising source in the production of biofuel. However, the cost of production is still the main obstacle to develop of this type of source. Although there are many extensive studies on the requirements provided for the cultivation of the microalgae, the study of the process, via the variables that affect the cultivation of microalgae, being still one of the important tasks to improve the production of biofuel. The present article is a serious attempt to investigate of use commercial fertilizer NPK (20:20:20+TE N: P: K) as considered a cheap nutrient medium in growth *Chlorella vulgaris* by comparison with traditional nutrient (Chu.10 medium). In addition, the current study addresses effect of different sparging periods of filtered air on the microalgae production. The experimental data showed that the use of the NPK fertilizer as cultivation medium in *Chlorella vulgaris* culture gives more growth rate of microalgae than that produced if the cultivation process was operated with Chu.10 medium. For example the maximum biomass concentration reaches to 0.3249 g L^{-1} when cultivated in NPK fertilizer, whereas reached to 0.212 g L^{-1} for cells cultivated in Chu.10 medium. In addition, the results proved that the aeration system in the cultivation can plays an important role in the activity of the microalgae with NPK medium, since it creates a convenient environment with low concentration of oxygen in the medium. The study showed that increasing aeration period for such a type of microalgae increases the growth rate.

Key words: Microalgae, *Chlorella vulgaris*, NPK, Aeration.

Introduction

Environmental and economic challenges are still facing the world due to use of fossil fuels as a main source of energy. The fluctuating rates in the prices as well as reaching the global thermal gases concentration to critical levels, has push the researches to find alternatives to this source [1-6].

Numerous attempts have been made to mitigate greenhouse gases, including physical and chemical substance reaction-based CO_2 mitigation, however; these methods are either consumer of energy dramatically or unconvincing economically [7, 8].

Biological treatments for fixing the greenhouse gases may be the best solution so far, such as carbon dioxide consumption by microalgae as a source of carbon and to adjust pH value [9].

This types of plants have ability for CO_2 biofixation efficiently by converting it (with light) into biomass and other secondary metabolites such as carbohydrates, lipids, chemicals, foods, feed , intracellular polysaccharides, proteins, pigments and other feed stock [10, 11]. Also, the microalgal has a higher photosynthetic efficiency, higher biomass production, faster growth related to other energy crops and they can grow anywhere without competing food crop [12-14]. Moreover, they need just few days to complete their growth cycle and their reproduction significantly [15].

There are an extensive attempts on the requirements provided for the cultivation of the microalgae, however; still needed to more studied by voice variables that affect directly or indirectly on the cultivation. The present study is one of these attempts to find the most favorable conditions to secure reasonable growth rate and biomass productivity for *Chlorella vulgaris* via the suggested hypothesis that says that uses a commercial fertilizer 20:20:20+TE NPK, as low cost nutrient and available, under aeration at different sparging periods can play an important role growth rate and productivity of *Chlorella vulgaris.*

Material and Methods

Microorganism and Culture Medium

Chlorella vulgaris was suggested as a microalga for this investigation, which belongs to the Chlorophyta group (freshwater green algae). This spece was originally isolated and purified at Plant Laboratory for Graduate Studies, department of biology, college of science, University of Baghdad by using serial dilution then different plating techniques as spread and streak method were carried out to purify the culture [16].

As shown in Figure 1 the snapshot of *Chlorella vulgaris* used in the present study using Microscope (CX21FS1, 40X, TOKYO, JAPAN) incorporated with a 20x canon camera.

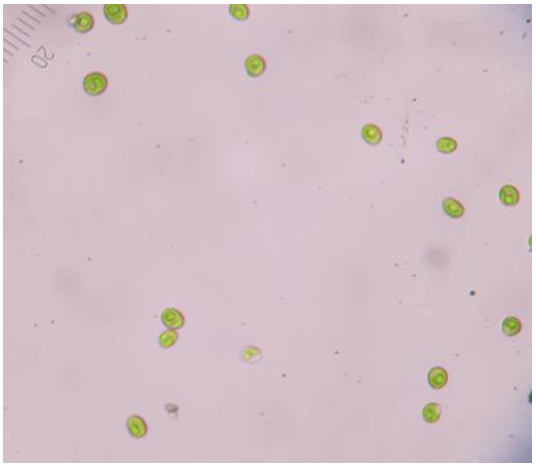

Fig.1: Microscopic photograph of the microalgae *Chlorella vulgaris* used in the present study using microscope (CX21FS1, 40X)

The suggested nutrient medium in this study was NPK medium (20:20:20+TE N:P:K) commercial fertilizer, which has the 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%. While the Chu-10 consisted of 40 (mg/l) Ca $(NO_3)_2$, 25(mg/l) $MgSO_4$, 5(mg/l) K_2HPO_4, 20 (mg/l) Na_2CO_3, 25(mg/l) Na_2SiO_3, 8(mg/l) $FeCl_3$, Both medium were prepared by dissolved these salts in RO water, while, the value of initial pH was adjusted to 6.23 using (0.1 N) of sodium hydroxide and hydrochloric acid.

Preparation of Microalgae Inoculum

A stock solution of *Chlorella vulgaris* was incubated in 500 mL conical flask in an environmental growth chamber at (25 ± 2 °C)

temperature and under continuous illumination by three cool-white fluorescent light tubes (10 watt and 10 inches length). Agitation system was obtained by bubbling filtered atmospheric air in the bottom of the flask. The medium used in the cultivation of the microalgae was Commercial available N: P: K 20:20:20+TE fertilizer (provided from Kule® Inc.). Advantages of this suggested nutrient are high water soluble and cheaper nutrients for biomass production. After seven days of inoculation, microalgae cells pre subculture by transferring it to fresh medium. Figure 2 shows the starter culture of *Chlorella vulgaris* microalgae in flasks at different times.

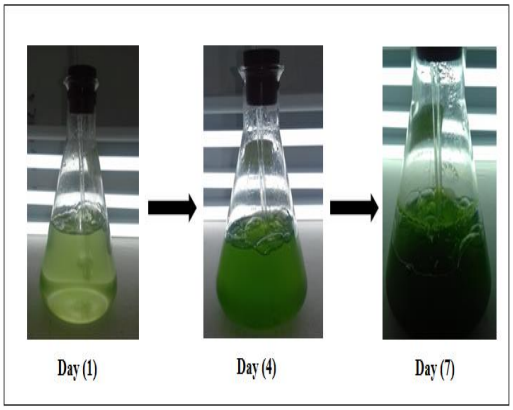

Fig. 2: Photographic View of the batch cultivation for *Chlorella vulgaris* with time

Experimental Setup and Measurements

The experiments of cultivation *Chlorella vulgaris* at different aeration periods were conducted in four conical flasks of 0.5 L capacity, operating in batch culture with a working volume of 350 ml. Each flask enclosed by stopper with two pores one for aeration and other for air exhaust. Air was supplied via air pump (HX-106A). The inlet air was dried by filtration through an in-line filter before entering the culture flasks to avoid contamination by condensation in the air tube airlines. This filter consists of cotton and activated charcoal that are arranged in layers.

To minimize the contamination problems or infection that can be causes by potential pathogens and other microorganism, the flasks and nutrient media were sterilized. The procedure of sterilization was carried out corroding to Ammar [17] using a water bath (Julabo, model: EH (V.2), Germany) with hot water at a temperature of (70-80 °C) for 20 minutes, as shown in Figure 3.

Fig. 3: A sterilization of nutrients media by using water bath

In each flask, 15 ml of culture was added and then completed to 350 ml working volume with fresh media solution for 14 days at room temperature (25 ± 2 °C) and under photoperiod artificial light (20 hr light/4 hr dark).

Agitation system was conducted by bubbling filtered air at bottom of the flask with different periods; 20hr, 6hr, 2hr and zero hour. All flasks were shaken manually thrice a day to meet their oxygen demands and keep the cell suspension for un-interrupted uniform multiplication of algal cell.

Samples of culture media were collected aseptically every 24hr intervals by taking a sample 5 ml of culture in 10 ml capacity vials to evaluate microalgae growth. The microalgae growth was determined by measuring the optical density (cell absorbance) with wavelength 680 nm [18, 19], using UV spectrophotometer

(GENESYS 10UV, USA) to ensure an exponential phase of growth and was maintained until it reached the stationary phase and correlated to the dry weight by a calibration curve. While the cell dry weight (biomass concentration, g L^{-1}) was determined by using centrifuge (PLC- 03, Taiwan) at 3000 r/min for 20hr and drying by exposure to atmosphere for 24 hr, then at $60C^0$ until constant weight. Six culture samples were tested and calibration curve for relationship between optical density and cell dry weight was determined.

For comparison study between N: P:K fertilizer and Chu-10, two batch mode in 500ml conical flasks were used. Value of pH was adjusted at 6.23 using (0.1 N) of sodium hydroxide and hydrochloric acid. Ten mile of green *Chlorella vulgaris* was added to each flask and completed to 450ml medium and were placed in environmental cultivation chamber.

Kinetic Parameters

The biomass concentrations (X, g L^{-1}) that estimated via calibration curve were used to construct growth curve of biomass density versus time to determine the specific growth rates (μ, d^{-1}), doubling time (td, d), maximum biomass concentration (X_{max}, g L^{-1}) and volumetric biomass productivities (P, g L^{-1} d^{-1}).

The specific growth rate, μ (day^{-1}) was estimated from Equation 1 [20]

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

Where X_t and X_0 are the final and initial dry biomass concentrations (g L^{-1}), respectively during the exponential logarithmic growth phase and Δt is the cultivation time in day during the exponential logarithmic growth phase [21].

While, the doubling time (td, d) was calculated from Equation 2 [9, 22].

$$\text{td} = \frac{Ln\ 2}{\mu} \quad \ldots (2)$$

Biomass productivity, P (dry g L^{-1} day^{-1}) in batch mode was calculated from the variation in biomass concentration within the cultivation time (day) according to Equation 3 [23].

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

Where X_t is the dry biomass concentration (g L^{-1}) at t (day) and X_0 is the dry biomass concentrations at inoculation [9, 22].

Result and Discussion

Comparison Study

The growth of the microalgae cells was estimated by optical density (Cell absorbance at 680 nm) every day. For counting, a 5 ml sample was aseptically removed from each culture using 10 ml capacity vials then correlated into dry weight by the liner regression y = 0.264 x. where's y is the biomass concentration (g L^{-1}) and x is the optical density (cell absorbance at 680 nm). Cell dry weight (biomass conc.) was measuring by weighted the cells after filtering and dried it at 60 °C for one hour. Then the growth curves of microalgae, specific growth rate (μ, d^{-1}), doubling time (td, d) and biomass productivity (g L^{-1} d^{-1}) were determined for each medium.

Figure 4 shows the characteristic of growth curves (lag, exponential, stationary and declining phases). It can be seen that the production of cell dry weight for the microalgae with NPK medium was more than that produced if the Chu-10 was used as a cultivation medium. In addition, with the N:P:K+TE fertilizer medium, the rate

of growth reached the peak on the 11th day, while in the Chu.10 medium the rate of growth reached the peak on the 14th day. The maximum biomass concentration reaches to 0.3249 g L^{-1} when cultivated in NPK fertilizer, whereas reached to 0.212 g L^{-1} for cells cultivated in Chu.10 medium. Figure 5 shows the Photographic View of the batch cultivation using NPK and Chu-10 medium.

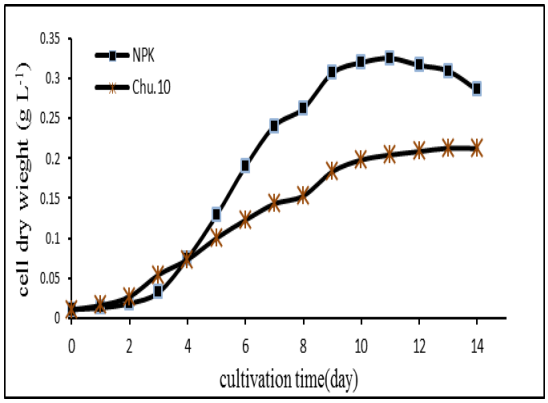

Fig. 4: the growth curve of *Chlorella vulgaris* cultivation in Chu.10 and NPK commercial fertilizer

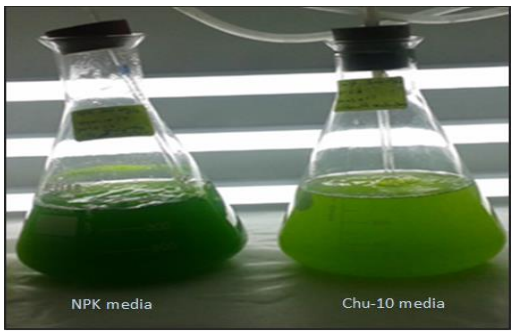

Fig. 5: Photographic View of the batch cultivation using N: P: K+TE fertilizer media and Chu-10 media

Maximum specific growth rate (μ, d^{-1}) was also 0.375 d^{-1} and 0.249 d^{-1} for NPK fertilizer and Chu.10 medium respectively. Biomass doubling time (td, d) was 1.8 day for NPK fertilizer while 2.7 day for Chu.10 medium as indicated in Figure 6. While the productivity of *Chlorella vulgaris* cells was 0.0328 g L^{-1} d^{-1} obtained in NPK fertilizer and 0.019 g L^{-1} d^{-1} obtained in Chu.10 medium.

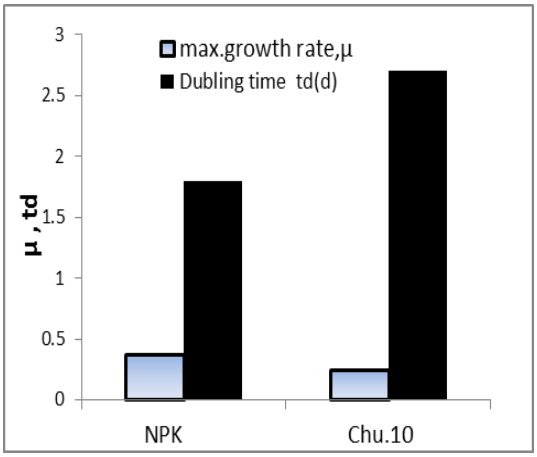

Fig. 6: Specific growth rate and doubling time for *Chlorella* species in both medium

From above results, it can be seen that NPK fertilizer is preferred as cultivation media commercially and biologically. Therefore; the current study used the NPK medium in microalgae cultivation at different aeration periods. Since, the results in this article agree with most of the literature concerning the use of commercial agricultural fertilizers, on the fact that the commercial fertilizer preparations can be as effective as analytical grade reagent for microalgae cultivation. It is well known, however, that the composition of culture media not only affects the cell productivity, but also affects yield of specific products and cell composition [24-26].

Effect of Aeration System on Growth Rate of Microalgae

The mathematical relationship between the optical density (with wavelength 680 nm) and the cell dry weight of *Chlorella vulgaris* was determined by the linear regression, as shown in Figure 7.

$$Y = 0.264\,X \qquad \ldots (4)$$

Where Y is the biomass concentration, which measured in (g L^{-1}), and X is the optical density (OD_{680} nm). The optical density was used to precisely predict the biomass concentration (R^2 >0.991; p <0.001).

Therefore, the measured values of optical density were used to calculate the biomass of *Chlorella* species in each experiment according the estimated equation in this study, to investigate the effect of aeration period by atmospheric air.

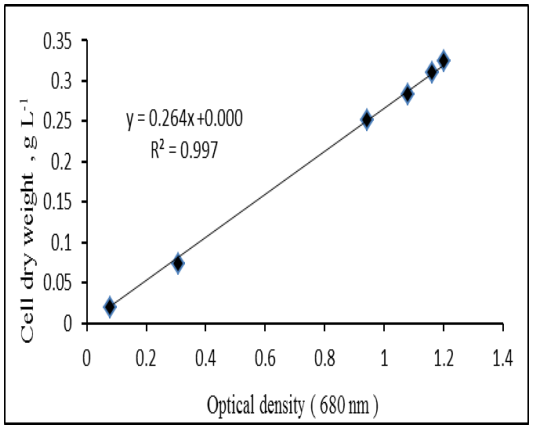

Fig. 7: Calibration curve for relationship between optical density and cell dry weight

In microalgae cultures, the growth curve has four growth phases. It starts with the lag or induction phase, which little increase in growth rate as cell density occurs. Then the logarithmic phase in which the growth rate increases exponentially by depending on many factors such as algal species or type, medium temperature and light intensity. In addition, the stationery phase is the constant phase in which the cell density become relatively constant. And finally death phase in which occur declining in growth rate when the cell divisions decreases because some factors become influential to growth rate such factors as nutrients medium concentration, dissolved CO_2 and O_2, pH, light and contamination risk. The present paper studied these phases through the growth curves as shown in Figure 8.

From this figure, it can be seen that the curves have a close biomass concentration during the first two day (lag phase). However, there is a significant increase in the rate of growth when increasing aeration time, as can be seen with 20 hr per day aeration compared with other periods aeration (i.e.6 and 2 hours). Moreover the stationary phase at 9^{th} day comparable to other curves which have low growth and reach stationary phase at 11^{th} day ,11^{th} day and 8^{th} day when the culture aeration with 6 hr, 2hr and zero hr (control) per day respectively. As the cells grew up to plateau stage, the maximum biomass concentration (X_{max}, g L^{-1}) observed 0.32 g L^{-1} for 20 hr aeration ,0.262 g L^{-1} for 6 hr aeration, 0.152 g L^{-1} for 2 hr aeration and 0.069 g L^{-1} for control but the last concentration obtained with longer time than the first one.

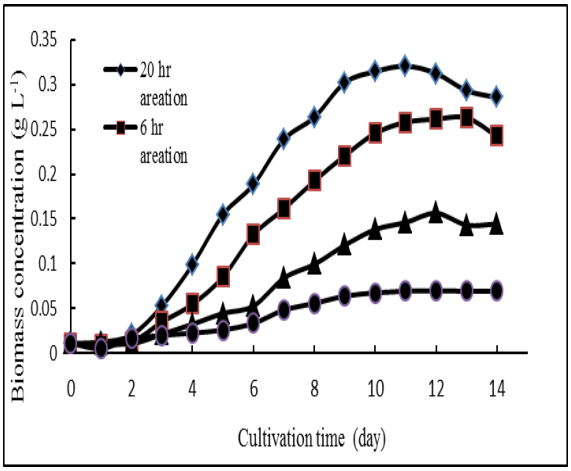

Fig. 8: Effects of different aeration time on the growth of *Chlorella vulgaris*

From these results we note that it agrees with the requirements of algae growth it needs to be ventilated as a source of carbon dioxide with light, nutrients and water needed for photosynthesis and note that in case of increased ventilation increases the efficiency of the process of photosynthesis and so increasingly turning inorganic carbon to Organic Carbon help of sunlight in with metabolism cellular. The photosynthesis process in microalgae chloroplast can offer reasonable explanation about importance of

aeration system in such of current process, since the synthesis of glucose for microalgae carries out using carbon dioxide and water according to Equation 5

$$6CO_{2(g)} + 6\,H_2O \longrightarrow C_6H_{12}O_6 + 6O_2 \quad \ldots (5)$$

The Gibbs free energy (ΔG) of the above reaction is + 2823 KJ mol^{-1} [27], thus this reaction is unspontaneous reaction thermodynamically. In fact the current process acts as chemical reaction which needs for enzymes as biocatalyst or external energy to occur. Otherwise, it is difficult of occurrence of above reaction within thermodynamic concepts, since it is endogenic reaction. However, the scenario of this process can be changed when the kinetic energy is supplied by molecular through the conversion and storage the absorbed light into ATP and NADPH2 form as shown in Figure 9 [28].

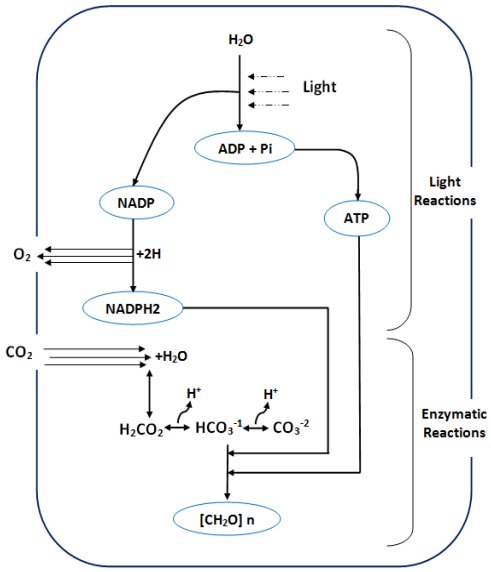

Fig. 9: Schematic mechanisms of photosynthesis in algae chloroplast

The supplied energy has a sufficient ability to break existing bonds and formation new bonds. Several enzyme catalyzed reactions may possibly progress in either the forward or opposite path way according to variation of light, temperature, concentration of reactant, and pH. Therefore and to evaluate the directionality of the biological reaction, ΔG that consider as one of the most essential application in thermodynamic, can be used to do work in biochemistry reaction [29].

The previous studies have applied these principals by removal of the products to make spontaneous reaction and to produce more products such as production of biohydrogen and carbon dioxide from glucose via specific bacteria. Park et al. [30] found that the overall H_2 production increased here by 43% with chemical scavenging of the carbon dioxide concentration in the head space of the reactor due to reduction of partial pressure of H_2 and CO_2. Alshiyab et al. [31]; and Tanisho et al. [32] demonstrated also that the removal of carbon dioxide by sparging the reactor with inert gas such as nitrogen leads to increase the H_2 production. Other researcher found similarity result such as Mizuno et al. [33] investigate that sparging culture with N_2 gas make H_2 increased by 68%, and found that hydrogen yield 1.43 mole /mole glucose under N_2sparging. Kraemer and Bagley [34] found the optimum N_2 sparging at a rate 12ml (min. L-liquid)$^{-1}$ maximised the yield of hydrogen at approximately (2 mole H_2 / mole glucose), versus1mole H_2/ mole glucose when no N_2 sparging. Liang et al.[35] also indicate increasing in H_2 production at about 15% due to the reducing partial pressure of H_2, when remove the dissolved gasses by using silicon rubber membrane. Therefore, the directionality of a reaction is be estimated merely by the concentrations of the products and reactants that are existent, this applied for biochemical processes that happen at constant

temperature and pressure. The important spontaneous reaction in biological system is the hydrolysis of ATP.

The current results supported this principle; since decreasing the concentration of the product (e.g.O_2) caused the reaction proceed to the forward direction as shown. Hence the reaction comes to be more thermodynamically promising and move towards to the producing further products.

The maximum specific growth rate (μmax, d^{-1}) and biomass doubling time (td, d) at each different aeration time can be calculated and shown in Figure 10 and Figure 11 respectively. It is clear that the maximum value of growth rate (0.384 d^{-1}) and shortest doubling time (1.8 day) where reported for the cells aerated at 20 hr per day.

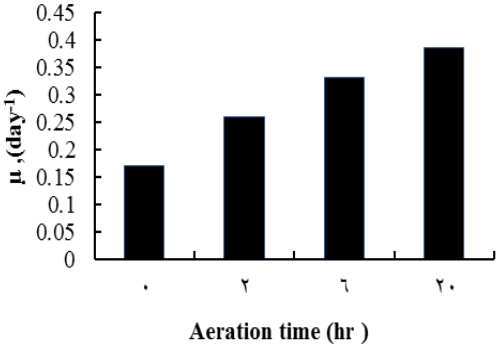

Fig. 10: Maximum specific growth rate for *Chlorella vulgaris* at different sparging time

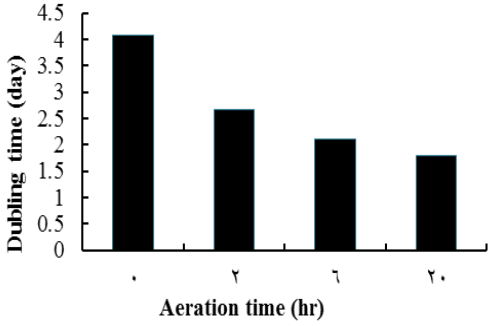

Fig. 11: Doubling time for *Chlorella vulgaris* at different sparging time

A notable increase in maximum growth rate can be observed in these cultures with longer aeration time compared to that lowest aeration time, as shown from Figure 10. The lowest maximum specific growth rate value (0.17 d^{-1}) for culture no aeration (control), while the maximum specific growth rate increased to around (0.348 d^{-1}) for culture aeration with 20hr. The maximum specific growth rate value for cultures aeration with 6hr and 2hr are (0.33 d^{-1}) and (0.266 d^{-1}) respectively.

In addition, the shortest biomass doubling time (td, d) was 1.8 day at 20 hr aerated while the longest td was 4 days at control and at 6hr and 2hr aeration were 2.1 and 2.6 days respectively as notice in Figure11. Therefore, as maximum growth rate increased, biomass doubling time decreases, and cultivation becomes more economically sustainable. Whereas microalgae can duplicate their biomass in less than 7 days, higher plants take many months or years [36]. In our study, the doubling time was equal to or less than 4 day.

Table 1 shows the main kinetic parameter (maximum specific growth rate μ_{max}, biomass doubling time td, maximum biomass productivity P_{max} and maximum cell concentration X_{max}) for culture *Chlorella* species at different aeration time.

Table 1: Kinetic parameters (μ_{max}, td, P_{max} , X_{max}) for *Chlorella vulgaris* at different aeration time

Aeration Time (hour)	**μ_{max} (day^{-1})**	**td (day)**	**P_{max} ($g\ L^{-1}.\ d^{1}$)**	**X_{max} ($g\ L^{-1}$)**
20hr	0.384	1.8	0.032	0.32
6hr	0.33	2.1	0.0234	0.262
2hr	0.266	2.6	0.0127	0.152
Control	0.17	4	0.0058	0.069

The productivity of *Chlorella vulgaris* (as a function of time) as

given in Table 1, increases with aeration period increase. It can be seen that the best *Chlorella vulgaris* obtained from the experiments that has a specific growth rate (μ_{max} = 0.384 d^{-1}), biomass doubling time (td = 1.8 day), maximum biomass productivity (P_{max} =0.0326 g L^{-1} d^{-1}), and maximum cell concentration (X_{max} = 0.32 g L^{-1}). All these results were obtained when the process was operated under 20 hr culture aeration. Previous studies have reported similar values, for example Blair et al. [37] and Gonçalves [38].They obtained 0.369 d^{-1} and 393 d^{-1} as a maximum specific growth rate as well as 0.038 g L^{-1} d^{-1} and 0.07 g L^{-1} d^{-1} as a biomass productivity respectively, although, they have used carbon dioxide gas in the aeration system as a main source for carbon and as a regulator of the pH.

From above it can be seen that the aeration system plays an important role in metabolic bioreactions via reduction the partial pressure of oxygen by removal of products gases (oxygen) to be more thermodynamically promising and move towards to the producing further products or as source of the carbon dioxide that found in air even with little percentage.

Conclusion

The current article suggested utilization of NPK fertilizer for bioprocess application (microalgae culture). In addition, different aeration periods were investigated in present study as well. The experimental data showed there is enhancement in growth rate of *Chlorella vulgaris* in commercial fertilizer NPK medium was more that with Chu.10 medium. For example, with the rate of growth reached the peak on the 11th day, while in the Chu.10 medium the rate of growth reached the peak on the ١4th day. The maximum biomass concentration reaches also to 0.3249 g L^{-1} when cultivated in NPK fertilizer, whereas reached to 0.212 g L^{-1} for cells cultivated in Chu.10 medium.

The present study proved that application of aeration system for the microalgae culture has a significant on growth rate, since the bioreactions become thermodynamically favorable and provide impetus for a higher level of production. Moreover, this article demonstrated that the production of *Chlorella vulgaris* increases when the sparging periods increase.

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Nomenclature

X	biomass concentration, (g L^{-1})
X_t	biomass concentration at any time t, (g L^{-1})
X_0	biomass concentration at the inoculation, (g L^{-1})
d	Time (day)
OD_{680}	optical density at 680 nm wavelength
td	doubling time (day)
Δt	cultivation time in day during the exponential growth phase
X_{max}	Maximum biomass concentration, (g L^{-1})
P_{max}	Maximum volumetric productivity, (g $L^{-1}d^{-1}$)
μ_{max}	maximum specific growth rate (day^{-1})

References

1. Ramanathan, V., (1988), "the Greenhouse Theory of Climate Change: a Test by an Inadvertent Global Experiment", Journal of Science, Vol. 240, PP. 293–299.
2. Song, C., (20060, "Global Challenges and Strategies for Control, Conversion and Utilization of CO_2 for Sustainable Development Involving Energy, Catalysis, Adsorption and Chemical Processing", Journal of Catalysis Today, Vol. 115, PP. 2–32.
3. Brown, L.M., and Zeiler, K.G., (1993), "Aquatic Biomass and Carbon Dioxide Trapping", International Journal of Energy Conversion and Management, Vol. 34, PP. 1005–1013.
4. Chang, E. H., and Yang, S. S., (2003), "Some Characteristics of Microalgae Isolated in Taiwan for Biofixation of Carbon Dioxide", Botanical Bulletin Academia Sinica, Vol.44, PP. 45- 52.
5. Le Treut, H., Somerville, R., Cubasch, U., Ding, Y., Mauritzen, C., Mokssit, A., Peterson, T., and Prather, M., (2007), "Historical Overview of Climate Change In: Climate Change 2007: The Physical Science Basis". Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, [Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L., (eds.)], Cambridge University Press, Cambridge, United Kingdom.
6. Hansen, J., Sato, M., Kharecha, P., Beerling, D., Berner, R., Mansson, V., Pagani, M., Raymo, M., Royer, D. L., and Zachos, J. C., (2008), "Target Atmospheric CO_2 Where Should Humanity Aim", Open Atmospheric Science Journal, Vol. 2, PP. 217-231.
7. Lin, C.C., Liu, W.T., and Tan, C.S., (2003), "Removal of Carbon Dioxide by Absorption in a Rotating Packed Bed", Journal of Industrial and Engineering Chemistry Research, Vol. 42, PP. 2381–2386.
8. IPCC (Inter-governmental Panel on Climate Change), (2001), "Climate Change 2001: Mitigation". Cambridge University Press, Cambridge.
9. De Morais, M.G. and Costa, J.A.V., (2007). "Biofixation of Carbon Dioxide by Spirulina Sp. and Scenedesmus Obliquus Cultivated in a Three Stage Serial Tubular Photo Bioreactor". Journal of Biotechnol, Vol. 129, PP. 439–445.
10. Sung, K.D., Lee, J.S. , Shin, C.S., Park, S.C. and Choi, M.J., (1999), "CO_2 fixation by Chlorella sp. KR-1 and its cultural characteristics", Journal of Bioresource Technology, Vol. 68, PP. 269- 273.
11. Babcock, Jr. R.W., Malda, J., and Radway, J.C., (2002), "Hydrodynamics and mass transfer in a tubular airlift photobioreactor", Journal of Applied Phycology, Vol. 14, PP. 169–184.
12. Dote, Y., (1994), "Recovery of Liquid Fuel from Hydrocarbon-Rich Microalgae by Thermochemical Liquefaction", Fuel, Vol. 73, PP. 1855–1857.
13. Miao, X.L., and Wu, Q.Y., (2006), "Biodiesel production from Heterotrophic Microalgal Oil", Journal of Bioresour Technology, Vol. 97, PP. 841–846.
14. Minowa, T., (1995), "Oil Production from Algal Cells of Dunaliella Tertiolecta by Direct Thermochemical Liquefaction", Fuel 74, 1735–1738.

15. Sheehan, J., Dunahay, T., Benemann, J., and Roessler, P., (1998), "A look back at the U.S. Department of Energy's Aquatic Species Program: biodiesel from Algae", National Renewable Energy Laboratory, USA.
16. Abed, I.J., AL-Hussieny, A.A., Kamel, R.F., and Jawad, A.M., (2014), "Environmental and Identification study of algae present in three drinking water plants located on Tigris River in Baghdad". International Journal of Advanced Research, Vol. 2, PP.895-900.
17. Ammar, S. H., (2016), "Cultivation of Microalgae Chlorella Vulgaris in Airlift Photo Bioreactor for Biomass Production using Commercial NPK Nutrients". Al-Khwarizmi Engineering Journal, Vol. 12, No.1, PP. 90-99.
18. Mohd, Y.A.Y, Hassan, J.M.B., Ashikeen, N. M., Sabuddin, R., Muda, R. A., Sulaiman, S., Makpol, S. and Wan, W.Z.N., (2011), "Fatty Acids Composition of Microalgae Chlorella Vulgaris can be Modulated by Varying Carbon Dioxide Concentration in Outdoor Culture". African Journal of Biotechnology, Vol. 10, No. 62, PP. 13536-13542.
19. Jeong, M.L., Gillise, J.M., and Hwang, J.Y., (2003), "Carbon Dioxide Mitigation by Microalgal Photosynthesis", Bull. Korean Chem., Vol. 24, No. 12.
20. Chiu, S.Y., Kao, C.Y., Tsai, M.T., Ong, S.C., Chen, C.H., and Lin, C.S., (2009), "Lipid Accumulation and CO_2 Utilization of Nannochloropsis Oculta in Response to CO_2 Aeration". Journal of Bioresour Technology, Vol. 100, PP. 833–838.
21. Ketheesan, B. and Nirmalakhandan, N., (2012), "Feasibility of Microalgal Cultivation in a Pilot-Scale Airlift-Driven Raceway Reactor", Journal of Bioresource technology, Vol. 108, PP. 196 - 202.
22. De Morais, M.G. and Costa, J.A.V., (2007). "Carbon Dioxide Fixation by Chlorella Kessleri, Chlorella vulgaris, Scenedesmus Obliquus and Spirulina Sp. Cultivate in Flasks and Tubular photobioreactors", Biochemical Lett. Vol.29, PP. 1349-1352.
23. Ryu, H.J., Oh, K.K., and Kim, Y.S., (2009), "Optimization of the Influential Factors for the Improvement of CO_2 Utilization Efficiency and CO_2 Mass Transfer Rate", Journal of Industrial and Engineering Chemistry, Vol. 15, PP. 471–475.
24. Sánchez, S., Martínez, M. E., and Espinola, F., (2000), "Biomass Production and Biochemical Variability of the Marine Microalga Isochrysis Galbana in Relation to Culture Medium", Biochemical Engineering Journal, Vol. 6, PP. 13-18.
25. Lourenço, S.O., Marquez, U. M. L., Mancini, F. J., Barbarino, E., and Aidar, E., (1997), "Changes in Biochemical Profile of Tetraselmis Gracilis I. Comparision of two Culture Media", Aquacultural Engineering Journal, Vol. 148, PP. 153-158.
26. Imamoglu, E., Sukan, E. F. V., and Dalay, M. C., (2007), "Effect of Different Culture Media and Light Intensities on Growth of Haematococcus pluvialis". International Journal of Engineering Science, Vol. 1, No. 3, PP. 5-9.
27. Haynie, D.T., (2008), "Biological Thermodynamics", Cambridge University Press, 2nd edition, P.137.
28. Lodish H., Berk A., Zipursky S. L., Matsudaira P., Baltimore D., and Darnell J., (2000), "Molecular

Cell Biology", 4th edition. W. H. Freeman and Company, New York.
29. Gary R. K., (2004), "The Concentration Dependence of the ΔS Term W in the Gibbs free energy Function: Application to Reversible Reactions in Biochemistry", Journal of Chemical Education, vol. 81, No.11, PP. 1599-1604.
30. Park, W., Hyun, S. H., Oh, S. E., Logan, B. E., and Kim, I S., (2005), "Removal of Headspace CO_2 Increases Biological Hydrogen Production", Journal of Environmental Science and Technology, Vol. 39, PP. 4416–4420.
31. Alshiyab, H., Kalil, M. S., Hamid, A. A., and Yusoff, W. M. W., (2008), "Removal of Headspace CO_2 Increases Biological Hydrogen Production by C. Acetobutylicu", Pakistan Journal of Biological Sciences. Vol.11, No.19, PP. 2336–2340.
32. Tanisho, S., Kuromoto, M., and Kadokura, N., (1998), "Effect of CO_2 removal on hydrogen production by fermentation", International Journal of Hydrogen Energy, Vol. 23 No.7, PP. 559–563.
33. Mizuno, O., Dinsdale, R., Hawkes, F. R., Hawkes, D. L., and Noike, T., (2000), "Enhancement of Hydrogen Production from Glucose by Nitrogen Gas Sparging", Journal of Bioresour Technology, Vol. 73, PP. 59–65.
34. Kraemer, J. T., and Bagley, D. M., (2008), "Optimization and Design of Nitrogen-Sparged Fermentative Hydrogen Production Bioreactors", International Journal of Hydrogen Energy, Vol. 33, PP. 6558–6565.
35. Liang T., Cheng, S., and Wu, K., (2002), "Behavioral study on hydrogen fermentation reactor installed with silicone rubber membrane", International Journal of Hydrogen Energy, Vol.27, PP. 1157–1165.
36. Vonshak, A., Abeliovich, A., Boussiba, A., Arad, S, and Richmond, A., (1982), "Production of Spirulina Biomass: Effects of Environmental Factors and Population Density", Biomass Journal, Vol. 2, PP.175–85.
37. Blair, M. F., Kokabian, B., and Gude, V. G., (2013), "Light and Growth Medium Effect on Chlorella Vulgaris Biomass Production ", Journal of Environmental Chemical Engineering, PP.1-10.
38. Gonçalves, A. L., (2012), "Environmental Benefits of *Chlorella Vulgaris* and Pseudokirchneriella Subcapitata CO_2 Capture and Bioenergy Production", MSc thesis, do Porto University.