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Comparison of Growth of *Chlorella vulgaris* in Flat-Plate Photobioreactor Using Batch, Fed-Batch, and Repeated Fed-Batch Techniques with Various Concentrations of Walne Medium

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ABSTRACT

Chlorella vulgaris was autotrophically grown in a Flat-Plate Photobioreactor (FPPB) using batch (BC), fed-batch (FB), and repeated fed-batch (RPF) cultivations. BC stage was done in the 20 L of working volume. During FB stages, 20 L of fresh medium was added to reach the final volume of 60 L. In the RPF stages, 20 L of broth was harvested, and then a similar volume of fresh medium was added to keep the working volume. This study aimed to evaluate the effect of Walne media (with various concentrations of composition) and cultural techniques on algal growth. Three different concentrations of Walne medium (original [coded as ×1], double [coded as ×2], and triple [coded as ×3] in concentrations of the original medium) was used for cultivations. Not only culture techniques but also various media affected biomass and chlorophyll productivity. RPF (with ×2 cultivation) gave the highest biomass productivity. However, RPF (with ×1 cultivation) gave the highest chlorophyll productivity. So, this study showed much fundamental information for the industrial production of *Chlorella vulgaris* biomass using flat-plate photobioreactors.

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1. Introduction

Microalgae are a diverse group of eukaryotic, aquatic, photosynthetic, and unicellular microorganisms. Microalgae contain nutraceutical elements, such as potassium, magnesium, zinc, iodine, selenium…Microalgae can convert solar energy, CO_2, and some nutrients into valuable biomass rich in proteins, lipids, and other organic compounds [1]. *Chlorella vulgaris* has attracted scientists for biomass production as a source of beneficial chemicals or health foods [2].

Microalgae cultivation can be applied in open-culture or closed-culture systems. Until now, a few microalgae species could be grown successfully commercially in an open-culture system. This system requires extensive land areas and is more susceptible to contamination from other organisms. So, a closed-culture system or a closed photobioreactor has been proposed, which possesses higher photosynthesis efficiency and could be easily controlled (e.g., temperature, nutrients). There are four main categories of photobioreactors: (a) tubular/horizontal, (b) column/vertical, (c) flat plate or flat panel, and (d) helical/tubular. The flat-plate photobioreactor (FPPB) has benefits including a wide lighting surface area, suitability for outdoor cultures, effectiveness in immobilizing algae, an excellent light path, high biomass productivity, affordability, ease of cleaning, ease of tempering, and low oxygen build-up [3]. Light availability is the main factor affecting microalgal growth in a photobioreactor [4].

In a fed-batch culture (FB), a batch culture (BC) is initially created, then an additional medium is introduced to raise the volume. The fermentation industry uses fed-batch culture to benefit from the fact that the limiting substrate concentration can be kept at a low level, avoiding the repressive effects. Basically, the removing a part of broth and using the leftover culture as the foundation for another fed-batch procedure; it called repeated fed-batch culture (RFP). The apparent benefit of repeated fed-batch culture is the ability to extend a process's productive phase under controlled circumstances. An additional benefit is the control of growth rate, which might present a chance to optimize product synthesis [5]. Theoretically, autotrophic or phototrophic cultivation is technically and economically

possible to produce large amounts of algal biomass. For the cultivation of microalgae, batch, fed-batch, repeated fed-batch, and continuous modes were investigated. Heterotrophic batch and fed-batch processes were applied to produce high biomass and lipid productivities of *Chlorella sp.* using stirred bioreactor [6]. A previous study reported the application of repeated fed-batch mode for photosynthetic *Spirulina platensis* cultivation in open raceway ponds [7]. Batch and continuous photobioreactors were used for *Chlorella vulgaris* mass production in mechanically agitated flasks [3]. *Chlorella vulgaris* biomass can be utilized for various purposes, such as: (1) producing biofuels, such as biodiesel or bioethanol, from its lipid or carbohydrate content, (2) producing food supplements or functional foods for human consumption or animal feed due to its high protein and nutrient content, (3)producing value-added by-products and bioactive compounds, such as antioxidants, pigments, polysaccharides, or proteins, (4) producing food supplements or functional foods for human consumption or animal feed due to its high protein and nutrient content, (5) treating wastewater from hydroponic or aquaponic systems by removing nutrients and pollutants, and (6) improving agricultural yield and soil quality by acting as a biofertilizer [8], [9].

This study observed the autotrophic growth of *Chlorella vulgaris* in a flat-plate photobioreactor using batch, fed-batch, and repeated fed-batch cultivations. Moreover, other Walne media concentrations were used in this study to investigate their effects on algal growth in these cultures (BC, FB and RFP).

2. Materials and Methods

2.1. Microalgae

The culture of *Chlorella vulgaris* was obtained from the collection of the Research Institute for Aquaculture No.2 (Ho Chi Minh City, Vietnam) and then was sub-cultured for four days in the original Walne liquid medium for the following experiments [10]. The seed culture (800 mL, from stock culture) was conducted in 1000 mL glass bottles. Filter air (0.2 µm) was continuously supplied (0.25 vvm) into the broth. The seed culture was finished after four days of cultivation.

The original Walne medium (coded as ×1 cultivation) was prepared as follows: (a) solution A (make up to 1000 mL with fresh water; 0.8 g $FeCl_3$, 0.4g $MnCl_2.4H_2O$, 33.6 g H_3BO_3, 45.0 g ethylene diamine tetra acetic acid as a disodium salt, 20.0g NaH_2PO_4, $2H_2O$, 100g $NaNO_3$); (b) solution B (make up to 1000 mL with fresh water; 2.1g $ZnCl_2$; 2.0g $CoCl_2$,6 H_2O, 0.9g $(NH_4)_6Mo_7O_{24}$, $4H_2O$, 2.0g $CuSO_4$, $5H_2O$, 10mL Concentrated HCl, make up to 100mL fresh water and heat to dissolve). Then, for the original Walne medium preparation, 1.0 mL solution A and 1.0 mL solution B were made up to 1000 mL with fresh water (heat to dissolve).

The primary cultivation was conducted in a flat-plate photobioreactor (100 cm long × 80 cm high × 10 cm wide). In batch cultivation (BC), seed culture of *C.vulgaris* (10%, v/v) was added to fresh original Walne medium in *FPPB* to reach an initial volume of 20 L. Filter air (0.2 µm) was continuously supplied (0.25 vvm) into broth through a linear porous sparger at the bottom of the photobioreactor. The *FPPB* was maintained at room temperature (~30^{o}C). The illumination was provided by 18W (~3000 lux) LED tube lamps (daylight type) at one side of *FPPB* during the experiment. At the end of batch culture (four days of BC cultivation), stages of fed-batch cultivation (*FB*) was subjected (coded as ×1, ×2, and ×3 cultivations which are mentioned below). Furthermore, after 4 stages of FB, stages of repeated fed-batch cultivation (RPF) were done (Figure 1-3).

In cultivation ×1, after 4 days of BC cultivation, when algal growth reached the end of the log phase, the cultivation changed to fed-batch mode (FB). 10 L of Walne medium (coded as ×1) was added to the FPPB. Then, 10 L of Walne medium was added to the FPPB until a total volume of 60 L was reached (FB1-FB4 stages). The time of media addition was determined when the growth of algae showed signs of ending the log phase (slowed growth rate). Then, at the end of the log phase of FB4 stage, 20 L of culture was removed, 20 L of medium (×1) was added, and the pH was adjusted to 7.0 (with concentrated H_2SO_4 solution) for conversion to the repeated fed-bat (RPF1) phase. Next, at the end of the RPF1 period, 20 L of culture was removed, and 20 L of medium (×1) was added to convert to RPF2 (no pH adjustment).

After 4 days of BC cultivation (×1 cultivation), the ×2 and ×3 cultivations were carried out (from FB1 to the end of processes). These cultivations used Walne medium with a 2-fold and 3-fold

concentration of components (compared to ×1 medium), respectively. Since the total culture time was 21 days, cultivation ×3 performed only RPF1 without RPF2. In addition, at the end of FB4 of the ×2 or the end of FB2-FB4 of the ×3 cultivations, because the pH tended to increase gradually and was higher than 9.0, logN and DCW showed signs of decreasing. Therefore, these broths were adjusted to pH 7.0 (with concentrated H_2SO_4 solution). In this study, other Walne media concentrations were used to investigate their effects on algal growth in different cultures (BC, FB and RFP).

For disinfection, the flat-plate photobioreactor and equipment were thoroughly cleaned with soap and clean water, 100 ppm chlorine solution, and sterilized water. Distilled and boiled water was used to prepare the medium. Furthermore, the photobioreactor was also cleaned with 75% ethanol and dried in direct sunlight before use.

2.2. Measurement of chlorophylls

The concentration of extracted chlorophyll was measured at 665 and 649 nm using a UV-Vis spectrophotometer (Bichrom Libra S32, USA). The concentration of chlorophyll a (C_a), chlorophyll b (C_b), and total chlorophyll (C_t) was calculated based on a published method [11]. Algae broth was centrifuged (6000 rcf, 15 min). The collected wet biomass (1.0 g) was supplemented with 5 mL of 96% ethanol and mixed thoroughly in the dark (15 min). The suspension was centrifuged (6000 rcf, 15 min) to collect the supernatant, which was then filled up to 100 mL. The chlorophyll solution was measured for absorbance at wavelengths 649 and 665. The formula calculated chlorophyll contents (mg/L):

$$Chlorophyll\ a\ (C_a) = 13.7 \times Abs_{665} - 5.76 \times Abs_{649} \quad (1)$$

$$Chlorophyll\ a\ (C_b) = 25.8 \times Abs_{649} - 7.60 \times Abs_{665} \quad (2)$$

$$Total\ chlorophyll\ (C_t) = 20.0 \times Abs_{649} - 6.10 \times Abs_{665} \quad (3)$$

2.3. Microalgal cell count

Cell density (logN) was counted using a hemocytometer (depth in 0.100 mm, 0.0025 mm^2, Hirschmann, Hirschmann Laborgeräte GmbH & Co.KG, Germany) under a light microscope with ×400 magnification [11]

2.4. Dried cell weight

The dried cell weight (DCW) of microalgae was estimated by centrifuging 40 mL of algal culture (6000 rcf, 15 min). Then, the pellet was washed with distilled water (10 ml) and centrifuged (6000 rcf, 15 min) twice. Washed cells were dried to constant weight at 80ºC in a vacuum oven. m_1, m_o, and V were the weight (mg) of the pellet before, after drying, and the sample volume (40 mL).

$$DCW\ (mg/L) = \frac{(m_1 - m_o)}{V} \times 1000 \quad (4)$$

2.5. Statistical analysis

Experiments were carried out in triplicate, with the mean values reported. All standard deviations (not shown) were less than 5% of the mean. ANOVA was used to examine the data, and Duncan's test (p <0.05) was used to establish the mean difference. SPSS software (Ver.17.0, SPSS, Chicago, IL, USA) was used for all statistical analyses.

3. Results and Discussion

Figure 1 showed the cell density and pH of the batch culture. In a batch culture (BC), the growth rate decreased from day 3-4 of the culture, and logN (7.13) barely changed. Thus, day four would be selected for the fed-batch cultivation (FB1) phase. Corresponding to the log phase, pH increased from 7.0 to ~8.7 after four days of culture. If culture was continued, the stationary phase would last for the next three days and then the death phase; respectively, pH would reach 9.0 on day five and rapidly decrease to 8.2 on day 9 (data not shown). That explained why FB1 would be conducted after 4 days of BC.

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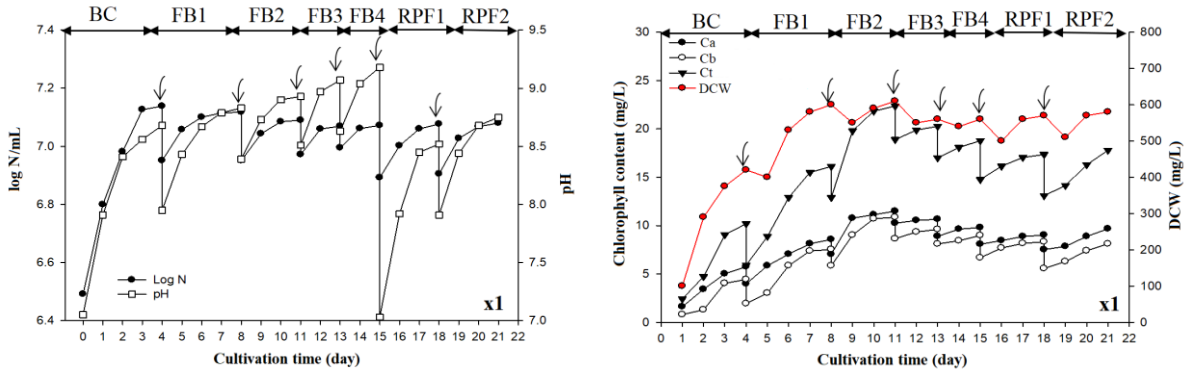

Figure 1. *Cell density (logN), pH, dry cell weight (DCW), and chlorophyll contents of ×1 cultivation.*

In cultivation ×1, FB1 and FB2 lasted the next four days with logN reaching 7.1. Correspondingly, the pH reached 8.7. The remaining FBs lasted the next 3, 2, and 2 days. As can be seen, the logN of FB2-FB4 reached ~7.05 and 9.0-9.2, respectively. In other words, logN tended to decrease and stabilize when conducting a fed-batch, while pH tended to increase gradually after four times of FBs. Following FB4, the repeated fed-batch phase continued. Compared with FBs, RPFs have similar logN but lower pH (about 8.5-8.7). Theoretically, without contamination, the RPF stages could last forever. Volumetric productivity (L/day) obtained for periods BC, FBs, and RPFs were 5, 4, and 6.67.

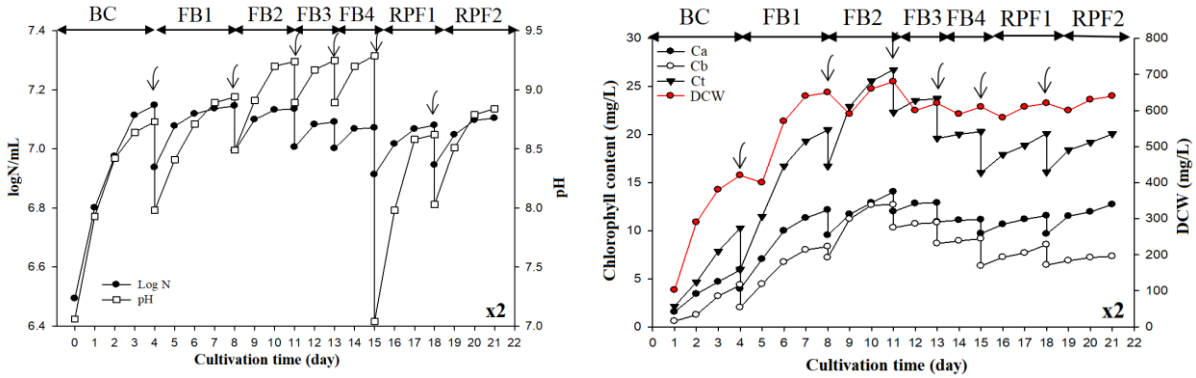

Figure 2. *Cell density (logN), pH, dry cell weight (DCW), and chlorophyll contents of ×2 cultivation.*

In ×2 and ×3 cultivations (Figures 2 and 3), logN, pH, DCW, and chlorophylls of the stages (BC, FBs, and RPFs) also occurred with a similar trend compared with that of ×1. Obviously, in FBs, the logN was arranged in the following order ×1 ≈ ×2 (7.1 logN/mL) < ×3 (7.18 logN/mL).

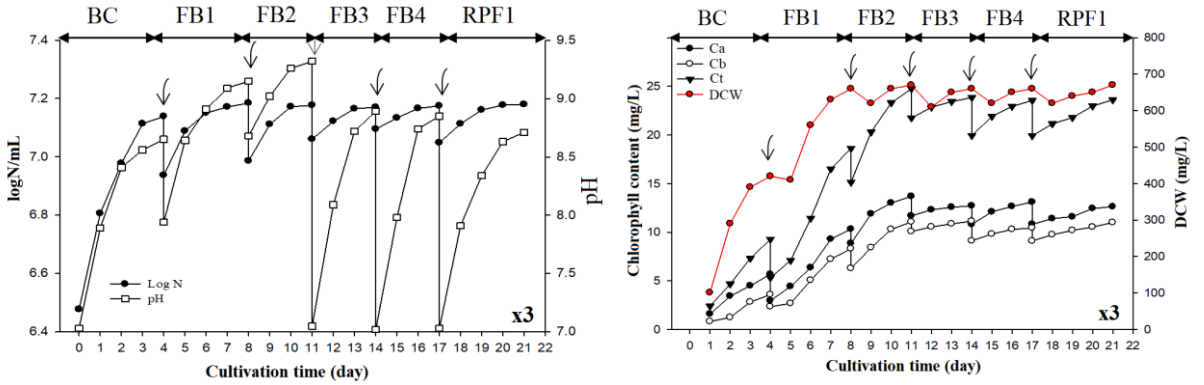

Figure 3. *Cell density (logN), pH, dry cell weight (DCW), and chlorophyll contents of ×3 cultivation.*

Generally, *DCW* productivity of modes of cultivation was ranked as RPFs > FBs > BC (Table 1). The highest *DCW* productivity was found in RPFs mode of cultivation ×2. It indicated that increased

medium component concentration can increase collected biomass. However, a medium with too high concentration (cultivation ×3) could significantly reduce *DCW* productivity. In addition, it can be observed (Table 1), FB3-FB4 of ×3 cultivation was one day longer than these stages of ×1 and ×2 cultivations. This can be explained by the high nutrient concentration creating a high osmotic pressure affecting algae growth.

Besides, the highest C_t *productivity* was found in FBs mode of cultivation ×1 (Table 1). The increase in medium component concentration resulted in a decrease in this productivity. Actually, it just the observation found in this study. Thus, further experiments and analytical measurements should be done to make clear this phenomenon. Obviously, both medium component concentration and mode of cultivation affected *DCW* and C_t productivities.

Table 1. *Productivity of dry cell weight (DCW, mg/day) and total chlorophyll content (C_t, mg/day)*[1,2]

Cultivation	Item	Modes of cultivation						
		BC	**FB1**	**FB2**	**FB3**	**FB4**	**RPF1**	**RPF2**
(×1)	(1)cultivation time for collection (day)	4	8	11	13	15	3	3
	(2)volume of collected broth (L)	20	30	40	50	60	20	20
	(3)*DCW* (mg/L)	412	598	612	563	560	569	579
	(4)C_t (mg/L)	11	17	25	20	18	17.5	17.6
	DCW productivity (mg/day)= (2)×(3)/(1)	$2{,}060^{a,A}$	$2{,}243^{a,E}$	$2{,}225^{a,C}$	$2{,}165^{B}$	$2{,}240^{D}$	$3{,}793^{F}$	$3{,}860^{G}$
	C_t (mg/day) productivity = (2)×(4)/(1)	55^{A}	128^{C}	333^{D}	500^{E}	540^{F}	117^{B}	117^{B}
(×2)	(1)cultivation time for collection (day)	4	8	11	13	15	3	3
	(2)volume of collected broth (L)	20	30	40	50	60	20	20
	(3)*DCW* (mg/L)	412	655	681	622	616	619	645
	(4)C_t (mg/L)	11	21	27	23.5	20.5	20	20
	DCW productivity (mg/day)= (2)×(3)/(1)	$2{,}060^{a,A}$	$2{,}456^{b,C}$	$2{,}476^{c,D}$	$2{,}392^{B}$	$2{,}464^{D}$	$4{,}127^{E}$	$4{,}300^{F}$
	C_t (mg/day) productivity = (2)×(4)/(1)	55^{A}	79^{B}	98^{E}	90^{D}	82^{C}	133^{F}	133^{F}
(×3)	(1)cultivation time for collection (day)	4	8	11	14	17	4	-
	(2)volume of collected broth (L)	20	30	40	50	60	20	-
	(3)*DCW* (mg/L)	412	662	665	661	660	663	-
	(4)C_t (mg/L)	11	18	24.5	24	23.5	23.5	-
	DCW productivity (mg/day)= (2)×(3)/(1)	$2{,}060^{a,A}$	$2{,}483^{c}$	$2{,}418^{b,B}$	$2{,}361^{B}$	$2{,}329^{B}$	$3{,}315^{C}$	-
	C_t (mg/day) productivity = (2)×(4)/(1)	55^{A}	68^{B}	89^{E}	86^{D}	83^{C}	118^{F}	-

[1]*For each mode of cultivation (each column), data of DCW (or C_t) productivities of different cultivations (×1, ×2, and ×3) were compared. Their significant differences were shown as different lowercase letters (a,b, and c).*

[2]For each cultivation (×1 or ×2 or ×3), data of DCW (or C_t) productivities of different modes of cultivation (each row) were compared. Their significant differences were shown as different uppercase letters (A, B, and C).

Theoretically, cell metabolism and biomass production are regulated by pH value [12], and a neutral pH favors microalgal growth. With the assimilation of CO_2 as the C source, the rapid growth of algae can cause the pH to rise. Additionally, the pH primarily influences the liquid chemistry of polar molecules and the availability of nutrients like iron, organic acids, and even CO_2 [12], [13]. Previous studies showed that the optimum pH for the growth of *Chlorella vulgaris* is around 7.0 – 8.0 [14], [15]. Furthermore, the concentration of CO_2 relates to the medium's pH value, and the consumption of CO_2 increases steadily in the medium [16]. During the cultivation, algae performed photosynthetic reaction to fix CO_2, inorganic carbon (HCO_3^-, CO_3^{2-}), and NO_3^- in the surrounding environment, which is assimilated leading to an increase in pH value [17]. Algae growth will be inhibited when the pH rises above the appropriate threshold (Figures 1 – 3). Obviously, in this study, pH greatly affected the growth of algae. Growth inhibition started at pH 9.0. At pH 9.5, growth was halted, leading to the appearance death phase. So, this rise in pH can be helpful in open ponds to neutralize pathogens in the treatment of microalgal wastewater, but it can also prevent the growth of microalgae [3]. Contamination has a significant impact on the commercial production of microalgal biomass. Eucaryotic species such as fungi, protozoans, big zooplankton, and filter-feeders contaminate microalgal cultures. Many control strategies, including heating, salinity, and the use of chemicals such as ammonium hydroxide, formalin, ivermectin, quinine sulfate, toosendanin, and rotenone, have been investigated [18]–[20]. Thus, maintaining a suitable alkaline pH of the microalgal culture can promote algal growth and limit contamination. In this study, the application of fed-batch and repeated-fed batch culture maintained a pH of 7.9 – 9.3, which could prevent contamination (Figure 1-3).

Basically, the photosynthetic process includes (i) photosystem I and (ii) photosystem II. Both chlorophyll a and chlorophyll b participate in these two systems. If the ratio of chlorophyll a:b is equal to 2, photosystem II plays a significant role; and if the ratio of chlorophyll a:b is 6, photosystem I play the primary role. Chlorophyll b is not involved in the formation of oxygen. Chlorophyll b is needed to efficiently use energy from light and help stabilize the photosynthetic system. The ratio of chlorophyll a:b increased with increasing light intensity [21]. Previous studies have shown that, in photosynthetic cultures, the cells use light energy either for maintenance purposes or to form new biomass [22]. As a result, biomass productivity and cell development rate are strongly related to the available light energy, which fluctuates from day to night. In this study, the ratio of C_a/C_b <2 shows that the light intensity is not optimal for the growth and development of *Chlorella vulgaris*.

A previous study used horizontal flat-plate (HFPP) and closed raceway-type (CRP) photobioreactors for C.vulgaris cultivation. For HFPP and CRP, the highest biomass concentration was 404 mg/L and 346 mg/L, respectively [23]. Another study showed that the maximum biomass concentration was 1000 mg/L [24]. Interestingly, the biomass concentration of *Chlorella vulgaris* reached 2500 – 4600 mg/L due to a CO_2 concentration of 1 – 4%. In this study, the maximum biomass concentration was around 700 mg/L. So, the biomass concentration varies based on medium type, substrate concentration, CO_2 concentration, photobioreactor type, the technique of cultivation, etc.

Therefore, further studies should focus on investigating the optimal lighting condition, medium type, CO_2 concentration, etc., for the growth of *Chlorella vulgaris*.

4. Conclusions

The above results showed that (1) the use of media with different nutrient concentrations and (2) the use of different culture techniques had a significant effect on the growth of *Chlorella vulgaris* in a flat-plate photobioreactor. As the concentration of Walne medium increased, growth also increased. However, when the medium concentration was three-time increased, the effect of osmotic pressure would reduced algae growth. In this study, the repeated fed-batch technique (×2 cultivation) produced the highest productivity of dry cell weight, and the batch technique had the lowest. Similarly, the productivity of total chlorophyll content obtained from fed-batch techniques (×1 cultivation) gave the best results. With the above repeated fed-batch technique, 20 L of algae can be obtained every three days, and the harvesting can take place for a long time without preparing a new batch. This dramatically reduces labor and equipment cleaning time and increases equipment efficiency. Many results were

observed in this study. However, this study should be continued with analytical measurements to clearly explain the results.

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Conflict of Interest

The author declares no conflict of interest.

REFERENCES

[1] S. K. Kim, *Handbook of Marine Microalgae: Biotechnology Advances*. Cambridge, MA, USA: Academic Press, 2015.
[2] Z. A. Khoeyi, J. Seyfabadi, and Z. Ramezanpour, "Effect of light intensity and photoperiod on biomass and fatty acid composition of the microalgae, Chlorella vulgaris," *Aquaculture Int.*, vol. 20, no. 1, pp. 41-49, 2012, doi: 10.1007/s10499-011-9440-1.
[3] G. A. Lutzu, "Analysis of the growth of microalgae in batch and semi-batch photobioreactors," Ph. D. dissertation, University of Cagliari, Cagliari, Italy, 2012.
[4] J. C. Ogbonna and H. Tanaka, "Light requirement and photosynthetic cell cultivation - Development of processes for efficient light utilization in photobioreactors," *Journal of Applied Phycology*, vol. 12, no. 3–5, pp. 207-218, 2000, doi: 10.1023/a:1008194627239.
[5] P. F. Stanbury, A. Whitaker, and S. J. Hall, *Principles of Fermentation Technology*, third ed. Oxford, U.K.: Elsevier, 2016.
[6] R. S. Coelho, A. D. S. Vidotti, É. M. Reis, and T. T. Franco, "High cell density cultures of microalgae under fed-batch and continuous growth," *Chem. Eng. Trans.*, vol. 38, pp. 313-318, 2014, doi: 10.3303/CET1438053.
[7] E. M. Radmann, C. O. Reinehr, and J. A. V. Costa, "Optimization of the repeated batch cultivation of microalga Spirulina platensis in open raceway ponds," *Aquaculture*, vol. 265, no. 1–4, pp. 118-126, 2007, doi: 10.1016/j.aquaculture.2007.02.001.
[8] I. T. K. Ru, Y. Y. Sung, M. Jusoh, M. E. A. Wahid, and T. Nagappan, "Chlorella vulgaris: a perspective on its potential for combining high biomass with high value bioproducts," *Applied Phycology*, vol. 1, no. 1, pp. 2-11, 2020. doi: 10.1080/26388081.2020.1715256.
[9] A. Pandey, D. J. Lee, Y. Chisti, and C. R. Soccol, *Biofuels from Algae*, Oxford, U.K.: Elsevier, 2014.
[10] P. Walne, "Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria, and Mytilis," *CCAP (Culture Collection of Algae and Protozoa)*, vol. 26, no. 5, pp. 0-62, 1970.
[11] M. Verawaty, E. Melwita, P. Apsari, and M. Wiyahsari, "Cultivation strategy for freshwater macro- and micro-algae as biomass stock for lipid production," *Journal of Engineering and Technological Sciences*, vol. 49, no. 2, pp. 261-274, 2017, doi: 10.5614/j.eng.technol.sci.2017.49.2.8.
[12] J. C. Goldman, Y. Azov, C. B. Riley, and M. R. Dennett, "The effect of pH in intensive microalgal cultures. I. Biomass regulation," *J. Exp. Mar. Biol. Ecol.*, vol. 57, no. 1, pp. 1-13, 1982, doi: 10.1016/0022-0981(82)90140-X.
[13] Y. K. Lee and S. J. Pirt, "CO_2 absorption rate in an algal culture: Effect of pH," *Journal of chemical technology and biotechnology. Biotechnology*, vol. 34, no. 1, pp. 28-32, 1984, doi: 10.1002/jctb.280340105.
[14] J. W. Rachlin and A. Grosso, "The effects of pH on the growth of Chlorella vulgaris and its interactions with cadmium toxicity," *Arch. Environ. Contam. Toxicol.*, vol. 20, no. 4, pp. 505-508, 1991, doi: 10.1007/BF01065839.
[15] C. Wang, H. Li, Q. Wang, and P. Wei, "Effect of pH on growth and lipid content of Chlorella vulgaris cultured in biogas slurry," *Chinese Journal of Biotechnology*, vol. 26, no. 8, pp. 1074-9, 2010.
[16] I. S. Suh and C. G. Lee, "Photobioreactor engineering: Design and performance," *Biotechnology and Bioprocess Engineering*, vol. 8, no. 6, pp. 313-321, 2003, doi: 10.1007/BF02949274.
[17] E. Granum and S. M. Myklestad, "A photobioreactor with pH control: Demonstration by growth of the marine diatom Skeletonema costatum," *J. Plankton Res.*, vol. 24, no. 6, pp. 557-563, 2002, doi: 10.1093/plankt/24.6.557.
[18] W. J. O'Brien and F. deNoyelles, "Photosynthetically Elevated pH as a Factor in Zooplankton Mortality in Nutrient Enriched Ponds," *Ecology*, vol. 53, no. 4, pp. 605-614, 1972, doi: 10.2307/1934774.
[19] J. J. Lee, M. E. McEnery, E. G. Kahn, and F. L. Schuster, "Symbiosis and the Evolution of Larger Foraminifera," *Micropaleontology*, vol. 25, no. 2, pp. 118-140, 1979, doi: 10.2307/1485262.
[20] Y. Wang, Y. Gong, L. Dai, M. Sommerfeld, C. Zhang, and Q. Hu, "Identification of harmful protozoa in outdoor cultivation of Chlorella and the use of ultrasonication to control contamination," *Algal Res.*, vol. 31, pp. 298-310, 2018, doi: 10.1016/j.algal.2018.02.002.
[21] B. J. Reger and R. W. Krauss, "The Photosynthetic Response to a Shift in the Chlorophyll a to Chlorophyll b Ratio of Chlorella," *Plant Physiol*, vol. 46, no. 4, pp. 568-575, 1970, doi: 10.1104/pp.46.4.568.
[22] S. J. Pirt, "The thermodynamic efficiency (quantum demand) and dynamics of photosynthetic growth," *New Phytologist*, vol. 102, no. 1, pp. 3-37, 1986, doi: 10.1111/j.1469-8137.1986.tb00794.x.
[23] D. E. O. Santiago, "Design and fabrication of flat-plate photobioreactors for microalgal biodiesel production," Rep. 868, 2012.
[24] A. M. Lakaniemi, V. M. Intihar, O. H. Tuovinen, and J. A. Puhakka, "Growth of Chlorella vulgaris and associated bacteria in photobioreactors," *Microb. Biotechnol.*, vol. 5, no. 1, pp. 69-78, 2011, doi: 10.1111/j.1751-7915.2011.00298.x.

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