Autoflocculation of *Scenedesmus Quadricauda*: Effects of Aeration Rate, Aeration Gas CO_2 Concentration and Medium Nitrogen Concentration

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Abstract

After microalgal cultivation step, a recent method to harvest microalgae is adding flocculant into microalgal liquid culture to cause flocculation. Flocculated cells are easy to concentrate since their sedimentation time and successive filtering time are expected to be shorter. In this sense, autoflocculation of microalgae may be a beneficial phenomenon for easing the harvesting process of microalgae. In this study, autoflocculation was investigated for the growth of *Scenedesmus quadricauda*, a type of fresh water microalgae, at 25 °C in a 5-L column photobioreactor. The BG11 medium was used. The effects of aeration rate, CO_2 concentration in aeration gas and nitrogen concentration in culture medium on auto flocculating behavior were investigated. Higher aeration rate, higher CO_2 concentration and higher nitrogen concentration caused more flocculation. In other words, autoflocculation became prominent at the condition range where the final cell biomass concentration was lower, in general.

Keywords: Autoflocculation, *Scenedesmus quadricauda*, aeration rate, aeration gas CO_2 concentration, medium nitrogen concentration.

1. Introduction

Recently, microalgae are considered as a potential feedstock for biodiesel with higher lipid accumulation and growth rate compared to terrestrial plants [1]. Additionally, microalgae have been utilized to capture CO_2 with 10 to 50 times efficiency higher than terrestrial plants because of more chlorophyll per unit area [2].

Many advantages of producing biofuel from microalgae have been pointed out [3]. However, the dilute nature of harvested microalgal cells creates a huge operational cost during harvesting. Thereby, microalgal fuel is less economically attractive. Besides, there is no proper method of harvesting for all species of microalgae. The choice of which harvesting method to apply will depend on the species of microalgae and the final product desired [4]. Microalgal cells harvesting is a major obstruction to industrial cultivation of microalgae for biofuel. Recently, the major methods which apply for the microalgal harvesting, include centrifugation, flocculation, filtration, gravity sedimentation, flotation, and electrophoresis techniques [5].

Flocculation happens when the cationic polymers are added into the microalgae culture [6]. In this sense, the microalgal cells are aggregated by the added flocculants and form the flocs which settle to the bottom [7]. The common flocculants include: multivalent metal salts, such as $FeCl_3$, $Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$, as well as polyelectrolyte flocculants [5].

In an advance with flocculation, autoflocculation does not require flocculants. It is considered as an attractive alternative low cost method. A few researchers explained that autoflocculation is caused by the increase of pH value [8]. The others explained that autoflocculation is caused by the extracellular polymeric substances which are released by the cells themselves [9]. Carbon dioxide and nitrate may be promising factors which could control autoflocculation of microalgae since they are the two major raw materials for organics in microalgae.

In the present study, the *Scenedesmus quadricauda (S. quadricauda)* was used as a testing candidate for autoflocculation. The effect of different aeration rates, aeration gas CO_2 concentrations and medium nitrogen concentrations on autoflocculation was investigated. The harvesting efficiency with autoflocculation and non autoflocculation were also pointed out.

2 Experimental

2.1 Microalgal Strain and Cultivation Conditions

The *S. quadricauda* strain was obtained from Algaetech International Sdn Bhd (Technology Park Malaysia, Kuala Lumpur, Malaysia). All the experiments were conducted with 5 L column photobioreactor (30 cm high, 16 cm internal diameter), presented in Figure 1, by BG-11 medium as shown in Table I.

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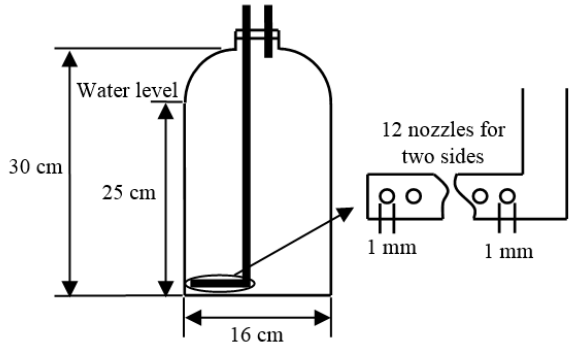

Figure 1 Schematic drawing of column photobioreactor

Table 1 The Composition of BG-11 Medium

Solution		Concentration (g/L)
$NaNO_3$ (Nitrogen source)		1.5
K_2HPO_4		0.04
$MgSO_4 \cdot 7H_2O$		0.075
$CaCl_2 \cdot 2H_2O$		0.036
Citric acid		0.006
Ferric ammonium citrate		0.006
EDTA (disodium salt)		0.001
Na_2CO_3		0.02
Trace metal	H_3BO_3	2.86×10^{-3}
	$MnCl_2 \cdot 4H_2O$	1.81×10^{-3}
	$ZnSO_4 \cdot 7H_2O$	0.222×10^{-3}
	$NaMoO_4 \cdot 2H_2O$	0.39×10^{-3}
	$CuSO_4 \cdot 5H_2O$	0.079×10^{-3}
	$Co(NO_3)_2 \cdot 6H_2O$	49.4×10^{-6}
Initial pH (modify by HCl 0.5M and Na_2CO_3 0.5M)		7.1

2.2 Experimental Design

The experimental procedure was a sequence of stock inoculum preparation, preculture and main culture.

Stock inoculum preparation. An amount of 20 ml of *S. quadricauda* living culture purchased from Algaetech was inoculated in 1 L column photobioreator. The culture was maintained at 0.5 L/min of aeration, 25 °C, 10000 Lux of illumination, with BG-11 medium and cultured for 10 days. Then, the culture was recovered by centrifuge (Hermle Z206A; 4260 x g, 5 min) and then resuspended into 1 L of fresh BG-11 medium to prepare stock inoculum. The stock inoculum was stored under dark condition at 5 °C.

Preculture. An amount of 100 ml of stock inoculum was inoculated in the 5 L colume photobioreactor. The culture was maintained at 2.0 L/min of aeration, 25 °C, 10000 Lux of illumination, with BG-11 medium and cultured for 10 days.

Main culture. The preculture after 10 days cultivation was used as inoculum for main culture. An amount of 500 ml of preculture was inoculated and the initial biomass was 0.22-0.23 g/L. Table 2 shows the cultivation conditions for the main cultures. Five different aeration rates, four different CO_2 concentrations and five different nitrogen concentrations were applied to investigate the effect of CO_2 and nitrogen concentration. During the investigation, other parameters were constant. In Table 1, the standard nitrogen concentration in BG-11 medium is 1.5 g/L which is considered as control (1.0N-BG11). Other concentration of $NaNO_3$ of 0.075, 0.15, 0.45, 0.75 and 3 g/L was referred to as 0.05, 0.1, 0.3, 0.5 and 2.0N-BG11.

Table 2 Cultivation Conditions for the Main Cultures

Cultivation conditions		Value
Constant conditions	Temperature	25 °C
	Illumination	10 000 Lux
	Initial biomass concentration	0.22-0.23 g/L
	Medium	BG11
	Working volume	5 L
	Reactor	Column photobioreactor
	Aeration rate	2 L/min
	CO_2 concentration	0.04vol% (air)
	Nitrogen concentration	1.0N-BG11
Investigated conditions	Aeration rate	1, 2, 3, 4, and 5 L/min
	CO_2 concentration	0.04 (air), 1, 3, and 5vol%
	Nitrogen concentration	0.1, 0.3, 0.5, 1.0, and 2.0N-BG11

2.3 Autoflocculation evaluation

The autoflocculation was evaluated by eye inspection and recorded by the following four indices:

0: the cells in form of flocs are not detected, there are only the single cells in liquid and they are homogeneous.

1: the cells in form of flocs are very little compared to the single cells, it is very difficult to detect the flocs due to the green liquid surrounding that consists of single cells.
2: most of the cells are in form of flocs, the number of the flocs is remarkable compared to the number of the single cells, the flocs are still surrounded by the slightly green liquid that consists of the single cells.
3: There are only the flocs in the culture, the flocs are surrounded by clear liquid and there is no single cell in the liquid by eyes-inspection.

An illustration of four indices (index 0, 1, 2 and 3) is shown in Figure 2.

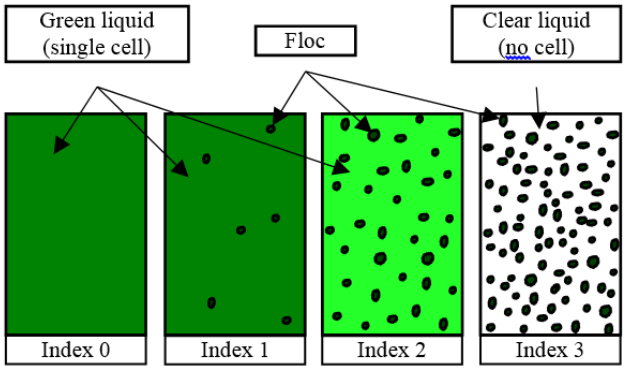

Figure 2 Four indeices of autoflocculation

3 RESULT AND DISCUSSION

In the present study, autoflocculation was investigated throughout the cultivation time even autoflocculation at the end of the log phase is effective for harvesting. The main reason of this is that it is worthwhile to observe the entire cultivation period to see the fundamental for auto flocculation.

3.1 Autoflocculation at Different Aeration Rates

Figure 3 shows the variation of autoflocculation depending on different aeration rates. The formation of the flocs started after one day of cultivation in most of the aeration rate except 1 L/min. There is no autoflocculation with 1 L/min during the whole cultivation time. Additionally, with the aeration rate at 2 L/min, the autoflocculation disappeared on the second day. With the aeration rate at 3 L/min, the autoflocculation was detected on the second day, however the autoflocculation disappeared on the third day. The significant difference was found in the cultures between higher aeration rate (4 and 5 L/min) and lower aeration rates (1, 2, 3 L/min). With 4, 5 L/min, the formation of autoflocculation on the first day is stronger than the formation with lower aeration rates. Besides, after the formation of the autoflocculation on the first day, the cultures with 4 and 5 L/min consisted only autoflocculation. And there is no single cell for the rest 12 days of cultivation.

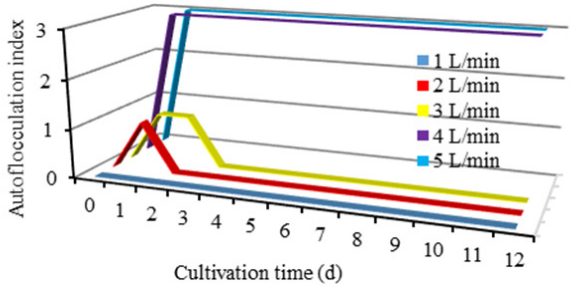

Figure 3 Autoflocculation during cultivation at different aeration rates

Figure 4 shows the culture samples with different aeration rates on the 12th day. Autoflocculation is not detected in the culture samples with 1, 2, 3 L/min. However, the flocs in the culture samples with 4 and 5 L/min is very obvious. In which, the flocs are surrounded by clear liquid containing no single cell by eye inspection.

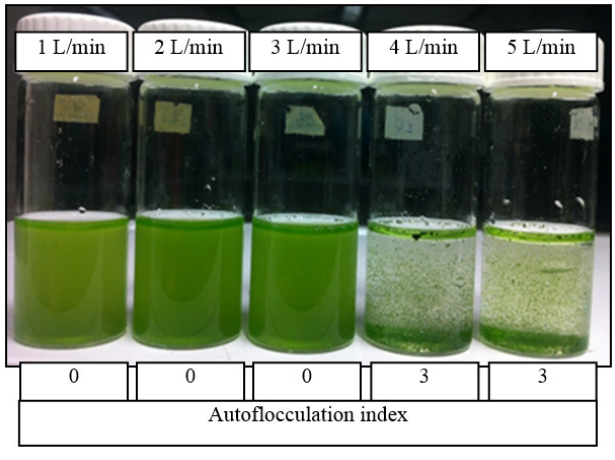

Figure 4 Culture samples with different aeration rates on the final day (12th day)

In the present study, the formation of autoflocculation is interesting since the autoflocculation was formed after one day of cultivation and enhanced with the higher of aeration rate. Besides, the strong formation and the durableness of the flocs with 4 and 5 L/min compared to lower aeration rates indicates that 3 L/min is the boundary of the autoflocculation stability.

Autoflocculation of microalgae happens when the microalgal cells have the ability to form the flocs spontaneously. There are only a few species showing the high autoflocculation ability

such as *Ankistrodesmus falcatus and Scenedesmus obliquus* [10]. Besides, in the research of Salim in 2012, *Ettlia texensis* was considered as the high autoflocculation which could create a fast sedimentation for harvesting [11].

3.2 Autoflocculation at Different Nitrogen and CO_2 Concentrations

The autoflocculation was also investigated under different nitrogen and CO_2 concentrations including 0.1, 0.3, 0.5, 1.0, and 2.0N-BG11 and 0.04, 1, 3 and 5vol% CO_2. Figure 5 shows the variation of autoflocculation with 0.04vol% CO_2 (air) depending on different nitrogen concentrations. With 0.04vol% CO_2, after one day of cultivation, a little autoflocculation appeared with all of the nitrogen concentrations. Within several days, the autoflocculation disappeared for all the cultures. Figure 6 shows the photos on the 19th day. Autoflocculation is not seen in all of the culture samples.

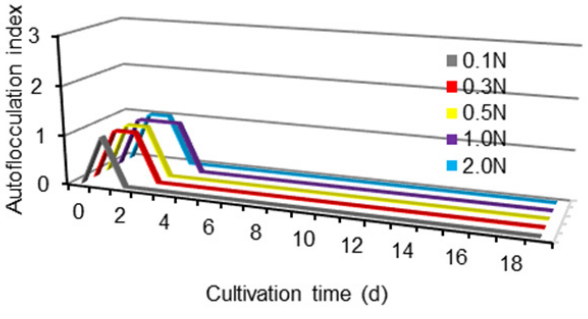

Figure 5 Autoflocculation during cultivation in 0.04vol% CO_2 (air) and different nitrogen concentrations

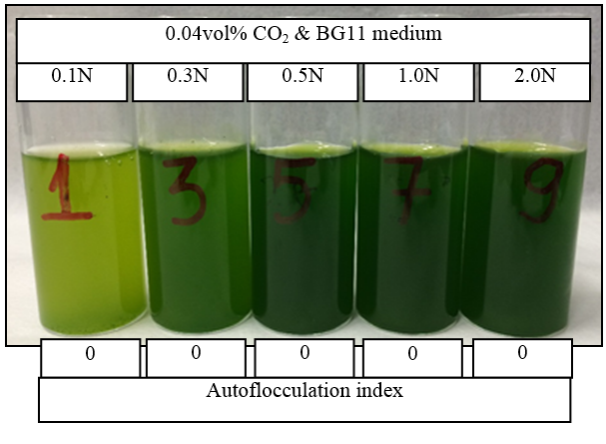

Figure 6 Culture samples with 0.04vol% CO_2 (air) and different nitrogen concentrations on the final day (19th day)

Figure 7 shows the variation of autoflocculation with 1vol% CO_2 depending on different nitrogen concentrations. Autoflocculation did not appear with low nitrogen concentration at 0.1 and 0.3N-BG11. However, the flocs formed with 0.5, 1.0, 2.0N-BG11 after one day of cultivation. Besides, the formation of the flocs with 1.0 and 2.0N-BG11 is stronger than with 0.5N-BG11. The flocs with 0.5N-BG11 only remained for 2 days and disappeared on the 3rd day. On the 2nd day, the occupation of the flocs with 1.0N-BG11 was less due to the increase of green liquid containing the single cells. Then, the condition of the autoflocculation with 1.0N-BG11 after 2 days was maintained similarly for the rest of the cultivation time (17 days). After the formation of the flocs, the flocs with 2.0N-BG11 remained strongly for the rest of cultivation time (17 days).

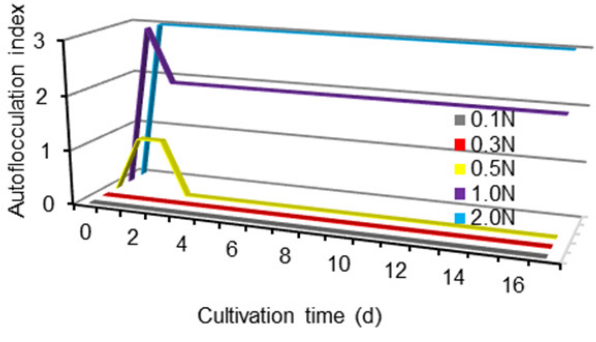

Figure 7 Autoflocculation during cultivation in 1vol% CO_2 and different nitrogen concentrations

Figure 8 shows the culture samples with 1vol% CO_2 and different nitrogen concentrations on the 17th day. Autoflocculation is not detected in the culture samples with 0.1, 0.3, 0.5N-BG11. However, the flocs are detected in the culture samples with 1.0 and 2.0N-BG11. Besides, the flocs in the culture sample with 2.0N-BG11 are surrounded by clear liquid containing no single cell. Consequently, the flocs in this culture sample are more obvious than 1.0N-BG11.

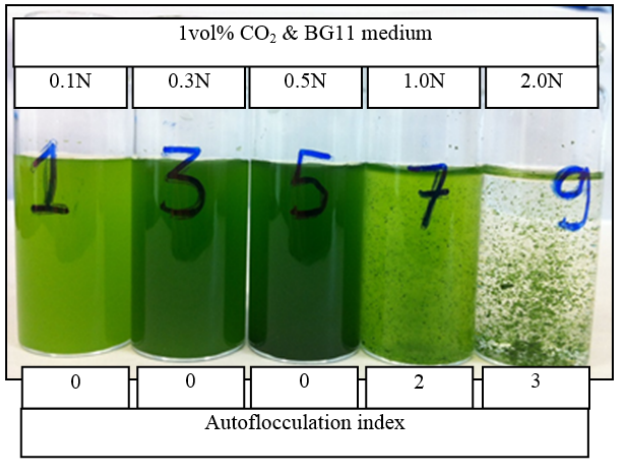

Figure 8 Culture samples with 1vol% CO_2 and different nitrogen concentrations on the final day (17th day)

Figure 9 shows the variation of autoflocculation with 3vol% CO_2 depending on different nitrogen concentrations. Autoflocculation did not appear with lower nitrogen concentrations at 0.1 and 0.3N-BG11 at all. However, the flocs were formed with 0.5, 1.0, 2.0N-BG11 after one day of cultivation. Besides, the formation of the flocs with 1.0 and 2.0N-BG11 was more prominent than that with 0.5N-BG11. After formation, the flocs with 0.5N-BG11 disappeared on the 2nd day. With 1.0N-BG11, from the 1st to 3rd day, the flocs reduced gradually. Then, the flocs disappeared on the 4th day. After the strong formation on the 1st day, on the 2nd day, the occupation of the flocs with 2.0N-BG11 was less than the 1st day due to the increase of green liquid containing the single cells. Then, from the 2nd to 20th day, the flocs and the single cells maintained the same condition, in which the flocs were surrounded by the slightly green liquid containing single cells.

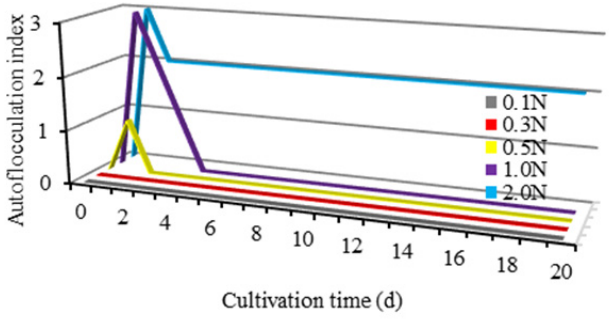

Figure 9 Autoflocculation during cultivation in 3vol% CO_2 and different nitrogen concentrations

Figure 10 shows the culture samples with 3vol% CO_2 and different nitrogen concentration on the 20th day. Only the culture sample with 2.0N-BG11 showed the Level 2 of autoflocculation.

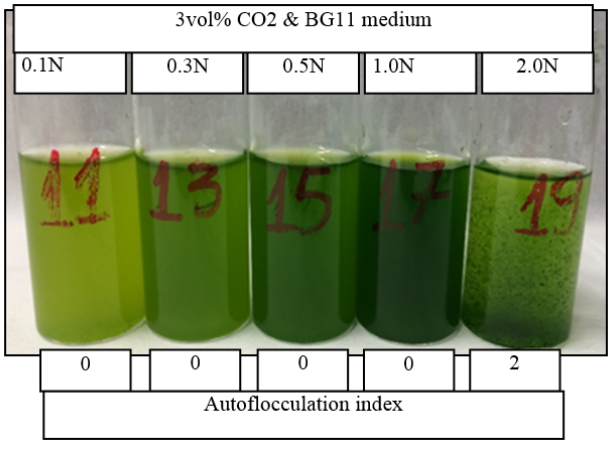

Figure 10 Autoflocculation during cultivation in 3vol% CO_2 and different nitrogen concentrations

Figure 11 shows the variation of autoflocculation with 5vol% CO_2 depending on different nitrogen concentrations. The effect of different nitrogen concentrations on the variation of autoflocculation is similar with that of 1vol% CO_2.

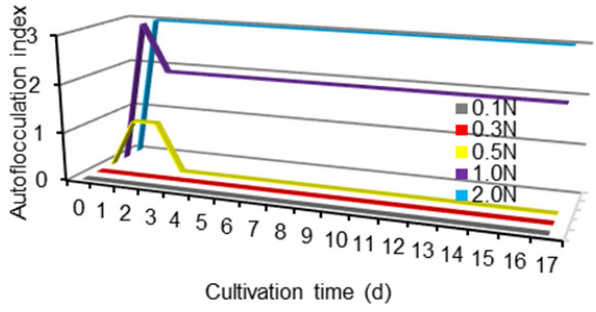

Figure 11 Autoflocculation during cultivation in 5vol% CO_2 and different nitrogen concentrations

Figure 12 shows the culture samples with 1vol% CO_2 and different nitrogen concentration on the 17th day. The effect of different nitrogen concentrations on the condition of flocs and single cell on the 17th day is similar between 5 and 1vol% CO_2.

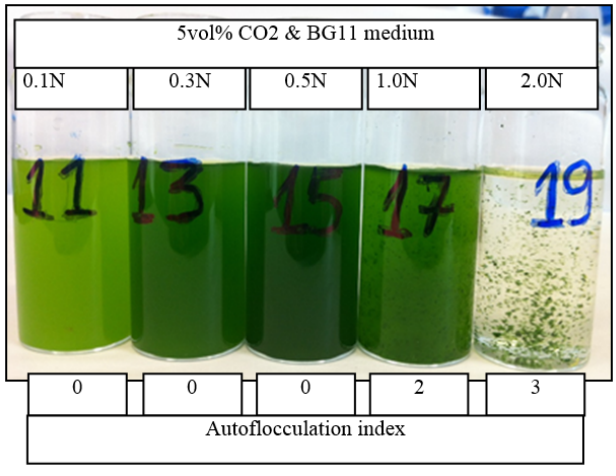

Figure 12 Culture samples with 5vol% CO_2 and different nitrogen concentrations on the final day (17th day)

At 0.04vol% CO_2, autoflocculation appeared only for a first few days and then disappeared. However, with the stimulation of CO_2 concentration, at 1, 3, 5vol% CO_2, in some cultures with higher nitrogen concentrations, autoflocculation appeared and was maintained strongly for the whole time of cultivation. Besides, for the effect of nitrogen concentration, the result shows that autoflocculation was enhanced significantly in the culture with high nitrogen concentration which was 1.0 and 2.0N-BG11. Autoflocculation appeared only a first few days with 0.5N-BG11. While, almost no autoflocculation was detected during the cultivation with 0.1, and 0.3N-BG11.

Recently, autoflocculation have been an attractive research topic because of its benefic in harvesting by sedimentation. The proposed reason for autoflocculation in the present study is related to the releasing extracellular polymeric substances (EPS) from the microalgal cells. In the study of Salim in 2014 on *Ettlia texensis*, the result of SEM analysis and the EPS extract showed that the main component of EPS was glycoproteins that patched in the cell surface and caused autoflocculation [11].

The possible mechanism of autoflocculation was also mentioned in the study of Salim in 2014. Microalgal autoflocculation was generated by specific interactions between microalgal cell membrane and the surrounding medium or between microalgae themselves. Spontaneously, microalgae released the EPS which mainly consisted of glycoproteins. The microalgal EPS can create a partial or completed binding with microalgal cells. With a partial binding, the unbinding part of EPS can bind to other microalgal cells, therefore the network of EPS and microalgal cells created the floc [9].

3.3 Autoflocculation an Advantageous Cells Harvesting Method

Figure 13 shows the sedimentation of 3 samples for 4 days. The 3 samples with the autoflocculation index of 0, 2, 3 were the culture samples on the final day (18th day) of 1vol% CO_2 with 0.5N-BG11, 1.0N-BG11, 2.0N-BG11, respectively. The sample with index 3 consists of only flocs which are surrounded by clear liquid containing no single cell. The sample with Index 2 consists of flocs which are surrounded by slightly green liquid containing single cells. The sample with Index 0 consists of strong green liquid containing single cell only. The sample with Index 3 takes only 5 min to sediment all of cells in form of flocs. Besides, in the sample with Index 2, flocs take 5 min to sediment, while the single cells still suspend in the liquid and only sediment all after 4 days. In the sample with index 0, after 30 min, the liquid does not change. Then, some cells sedimented after 12 hrs, and the sedimentation only completed after 4 days.

In the present study, the microalgal cells formed the floc without adding any flocculants and showed the fast sedimentation (only 5 min). An excellent harvesting method by autoflocculation was recommended in the present study. However, the cell biomass productivity in the cultivation conditions that has the autoflocculation was very low (data did not show). The possible reason for this low cell biomass issue can be distributed by the formation of the flocs. Whereby, only the cells at the surface of the floc can contact with light, CO_2 and nutrient. While, the cells inside the body of the floc were isolated from light, CO_2 and nutrient. Therefore, the growth of the cells inside the floc is very low. Besides, the number cells inside the body of the floc is higher than the cells at the surface of the floc. Consequently, the overall microalgal growth of the culture was very low when the cells formed the flocs.

The present study indicates that the cells grew very low in form of flocs. Thereby, the recommended cultivation should be carried out by avoiding autoflocculation. Then, for harvesting, the cultivation condition will be changed to the appropriate condition for autoflocculation formation. The flocs will formed and support the harvesting by fast sedimentation.

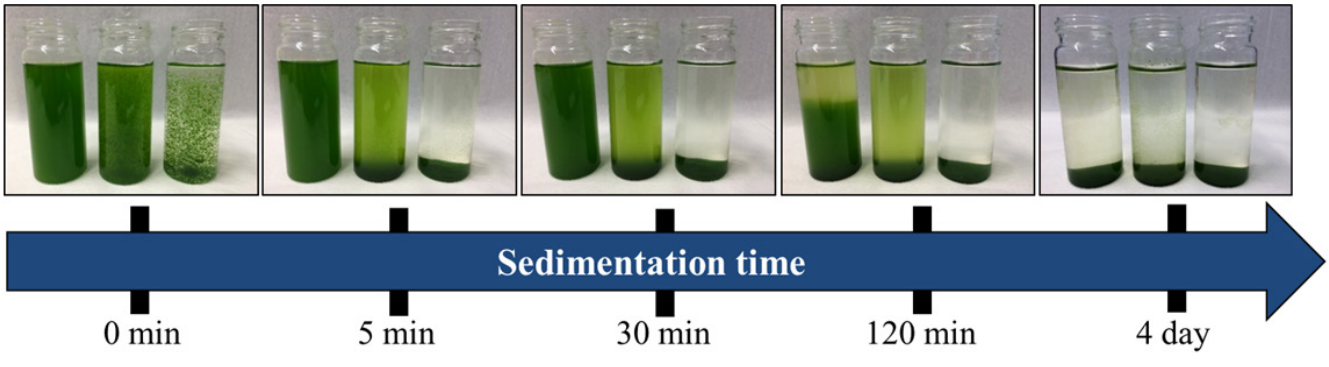

Figure 13 Sedimentation comparison among three different autoflocculation indices: 0 (left), 2 (center), 3 (right)

4 Conclusion

The autoflocculation formation is enhanced by higher aeration rate, higher CO_2 concentrations and higher nitrogen concentrations. Additionally, the culture sample with autoflocculation performed an impressively effective harvesting by very fast sedimentation with only 5 min and without adding any flocculants.

5 Acknowledgments

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References

[1] A. L. Ahmad, N. H. M. Yasin, C. J. C. Derek, and J. K. Lim, "Microalgae as a sustainable energy source for biodiesel production: A review," *Renewable and Sustainable Energy Reviews*, vol. 15, no. 1, pp. 584–593, Jan. 2011.

[2] M. F. Demirbas, "Biofuels from algae for sustainable development," *Applied Energy*, vol. 88, no. 10, pp. 3473–3480, Oct. 2011.

[3] L. Brennan and P. Owende, "Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products," *Renewable and Sustainable Energy Reviews*, vol. 14, no. 2, pp. 557–577, Feb. 2010.

[4] C.-Y. Chen, K.-L. Yeh, R. Aisyah, D.-J. Lee, and J.-S. Chang, "Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review," *Bioresource Technology*, vol. 102, no. 1, pp. 71–81, Jan. 2011.

[5] N. Uduman, Y. Qi, M. K. Danquah, G. M. Forde, and A. Hoadley, "Dewatering of microalgal cultures: A major bottleneck to algae-based fuels," *Journal of Renewable and Sustainable Energy*, vol. 2, no. 1, p. 012701, Jan. 2010.

[6] A. Papazi, P. Makridis, and P. Divanach, "Harvesting Chlorella minutissima using cell coagulants," *J Appl Phycol*, vol. 22, no. 3, pp. 349–355, Sep. 2009.

[7] I. Rawat, R. Ranjith Kumar, T. Mutanda, and F. Bux, "Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production," *Applied Energy*, vol. 88, no. 10, pp. 3411–3424, Oct. 2011.

[8] J.-I. Horiuchi, I. Ohba, K. Tada, M. Kobayashi, T. Kanno, and M. Kishimoto, "Effective cell harvesting of the halotolerant microalga Dunaliella tertiolecta with pH control," *Journal of Bioscience and Bioengineering*, vol. 95, no. 4, pp. 412–415, 2003.

[9] S. Salim, N. R. Kosterink, N. D. Tchetkoua Wacka, M. H. Vermuë, and R. H. Wijffels, "Mechanism behind autoflocculation of unicellular green microalgae Ettlia texensis," *Journal of Biotechnology*, vol. 174, pp. 34–38, Mar. 2014.

[10] S. Salim, M. H. Vermuë, and R. H. Wijffels, "Ratio between autoflocculating and target microalgae affects the energy-efficient harvesting by bio-flocculation," *Bioresour. Technol.*, vol. 118, pp. 49–55, Aug. 2012.

[11] S. Salim, Z. Shi, M. H. Vermuë, and R. H. Wijffels, "Effect of growth phase on harvesting characteristics, autoflocculation and lipid content of Ettlia texensis for microalgal biodiesel production," *Bioresource Technology*, vol. 138, pp. 214–221, Jun. 2013.