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Research Article

Utilization of Biogas as Carbon Dioxide Provider for *Spirulina platensis* Culture

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Abstract: The purpose of this study was to study the effect of biogas utilization as CO_2 provider to *S. platensis* growth rate. Two scenarios of culture was conducted in this study i.e., Run 1 = culture was supplied using air continuously and Run 2 = culture was supplied intermittently using biogas and air. The results showed that growth rate of *S. platensis* in Run 1 and Run 2 was $0.21*10^{-3}$ and $0.39*10^{-3}$/min, respectively. pH culture tend to decrease when supplied by biogas continuously. Kinetic model of *S. platensis* growth was modeled through modified Gompertz equation. The kinetic constants of Gompertz equation were obtained as follows: A (maximum value of OD_{680} reached), μ (maximum specific growth rate), λ (lag time) for Run 1 and Run 2 were 0.663; $0.459*10^{-3}$/min; 1454.9 min and 0.744; $0.588*10^{-3}$/min; 1024.5 min, respectively

Keywords: Biogas, CO_2, growth rate, kinetic model, *spirulina*

INTRODUCTION

Biomass of *Spirulina* contains fitonutrient and functional nutrient in large amount that have positive effect to human health (Henrikson, 2009). Naturally, *Spirulina* has low cholesterol, low fat and low calorie. In addition, *Spirulina* contains 9 important vitamins and 14 minerals that are bond with amino acid. Therefore, *Spirulina* can be assimilated easily by human body (Tietze, 2004). Based on that, *Spirulina*is one of the potential food sources in the future time.

Spirulina needs inorganic carbon (CO_2) for photosynthetic process. In the process of photosynthesis, *Spirulina* converts inorganic carbon (CO_2) into organic carbon with the help of light energy. Source of inorganic carbon can be obtained from synthetic nutrient such as $NaHCO_3$ (Hadiyanto and Hartanto, 2012; Cheunbarn and Peerapornpisal, 2010). Besides that, compressed CO_2 gas also can be used as inorganic carbon source (Becker, 1994). In other hand, utilization of $NaHCO_3$ and compressed CO_2 gas requires relatively large cost (Becker, 1994). Therefore, some authors investigated to find CO_2provider that is economically and environmentally (Van Den Hende *et al*., 2012). There are flue gas from power plant (Brown, 1996; Ho *et al*., 2011; Jacob-Lopes *et al*., 2010) and biogas from anaerobic digestion (Kao *et al*., 2012a, b; Mann *et al*., 2009).

At present, utilization biogas as CO_2 provider becomes the interesting study by authors. This concept has some advantages, which are:

- Purification of biogas, so that the heating value of biogas is up
- Cultivation of microalgae, because microalgae will uptake CO_2 from biogas to photosynthetic process and produce biomass
- Reduction in the cost of nutrient synthetic needs

Some authors studied cultivation microalgae using biogas as CO_2 provider. Kao *et al*. (2012b) used biogas that contained 20±2% CO_2 for *Chlorella sp.* culture with variation of light intensity which was at cloudy and at sunny day. Kao *et al*. (2012a) used biogas that contained 20±1% CO_2 for *Chlorella sp.* culture with variation flow rate of biogas which was 0.05; 0.1; 0.2; 0.3 vvm. Mann *et al*. (2009) used biogas that contained 42% CO_2 for *Chlorella vulgaris* with variation of light intensity which was 35; 60; 100 μmol/m^2.s. Douškova *et al*. (2010) investigated the potential of biogas as CO_2 provider for *Chlorella vulgaris.*

From information above, cultivation of *Spirulina* using biogas as CO_2 provider did not conducted yet by the other authors. Therefore, in this study, authors investigated the effect of aeration using biogas to growth rate and pH profile of culture. In view of *Spirulina* has big potential to be food source in the future time, this study was important to do.

MATERIALS AND METHODS

Preparation of microalgae. Microalgae used was *Spirulina platensis* obtained from the collection of C-BIORE University of Diponegoro, Indonesia. *S.*

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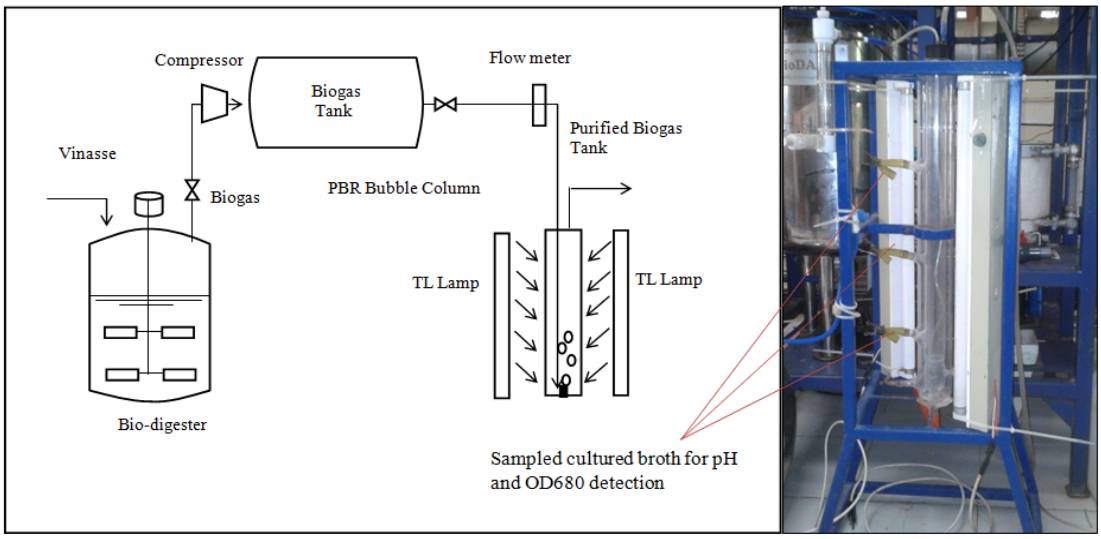


Fig. 1: Experimental set up of cultivation *S. platensis*

Table 1: Composition of biogas

Component	Value (%)
CH_4	42.37
CO_2	48.97
CO	1.92
The others	6.74

platensis was cultivated on medium that was developed by Hadiyanto and Hartanto (2012) with nutrients: 1 g/L $NaHCO_3$ (purity 98%), 0.05 g/L urea (46% N content), 10 ppm TSP (45% P_2O_5 content). Cultivation was done in room temperature. Artificial light as light source was obtained from tube light (TL) lamp 18 watt placed with distance of 10-15 cm from culture. Initial pH culture was adjusted 9.0 using HCl 1 M or NaOH 1 M. After cultivation 5-6 days, culture of *S. platensis* had OD_{680} value of 0.5~0.6. This culture condition was used to this study which was investigation of biogas utilization as CO_2 provider.

Preparation of biogas: This study used biogas that produced from vinasse using anaerobic digestion. Based on ours previous study, the composition of biogas can be seen in Table 1.

Experimental set up: Culture of *S. platensis* that had OD_{680}~0.6 putinto bubble column photo-bioreactor (PBR). PBR was designed with height of 64 cm and diameter of 4 cm. Material used to design PBR was acrylic polymer. Biogas was supplied from bottom of PBR with flow rate 100 mL/min. Artificial light as light source was obtained from Tube Light (TL) lamp 18 watt placed with distance of 10-15 from PBR. Experimental set up of this study can be seen in Fig. 1.

Experimental design: In this study, biogas was supplied for 4 h into culture. Optical Density (OD) of biomass was measured using spectrophotometry UV-VIS (SP-300) at wave length 680 nm each 60 min and pH culture was measured using pH meter each 20 min. Then culture was aerated using air for 6 h. OD_{680} and pH of culture were measurement each 60 and 20 min, respectively. Authors also did cultivation of *S. platensis* with aeration using air as comparison. Detail of experimental design can be seen in Table 2.

Experimental procedures. Optical density of all variables was measured two times by using spectrophotometry UV-VIS at λ 680 nm each 60 min. Value of pH culture was measured by using pH meter each 20 min. The results of investigation were used to calculate the growth rate, growth curve and pH profile curve:

$$\mu = \ln (ODi - OD0)/(ti - t0)$$

where,

μ, = Growth rate (/day)
OD_i = Optical density at t_i
OD_0 = Optical density at t_0
t_i, = Cultivated time i
t_0, = Cultivated time 0.

Kinetic model of *S. platensis* growth: Many authors studied about growth of microalgae, but they did not model it. Zwietering *et al*. (1990) stated that predictive modeling of microorganism growth allowed the prediction of shelf life of products, the detection of critical parts of the production, the optimization of production. Zwietering *et al*. (1990) proposed the modified Gompertz equation to make model of microorganism growth, which is written as:

$$y = A.exp.\left\{-\exp\left[\frac{\mu.e}{A}(\lambda - t) + 1\right]\right\}$$

Authors modeled growth of *S. platensis* using modified Gompertz equation above, with kinetic constant:

Table 2: Experimental design

Run	Aeration	Cultivation time	Response	Result
1	Air	10 h	OD_{680} and pH of culture were measurement	Effect of difference of aeration composition
2	Biogas-Air	4 h - 6 h	each 60 and 20 min, respectively	to OD_{680} and pH of culture

Remarks: OD_{680}, Optical density at wave length 680 nm; h, hours

y = OD_{680} at any time
A = Maximum value of OD_{680} reached
μ = Maximum specific growth rate (/minute)
λ = Lag time (minutes)
e = Mathematical constant (2.718282)

RESULTS AND DISCUSSION

Effect of biogas aeration to growth of *S. platensis* and pH culture: Comparison of *S. platensis* growth curve between on culture aerated using air and medium aerated using biogas can be seen in Fig. 2 and 3. Growth of *S. platensis* increased quickly at first 120 min and then decreased at second 120 min aeration using biogas (Fig. 3). The quickly growth at beginning cultivation was caused by availability of CO_2 that was in large amount in culture. However, continuous supplying of biogas that was rich CO_2 caused drop in pH culture. Carbon dioxide was reacted with water (H_2O) to form H_2CO_3 (Carbonate acid). In culture, H_2CO_3 was dissociated into HCO_3^- and H^+. Accumulation of H^+ ion caused pH culture drop. Meanwhile, HCO_3^- was changed by *S. platensis* with help carbonic anhydrase enzyme into CO_2 and OH^-. Carbon dioxide formed was used as inorganic carbon source for photosynthetic process and ion OH^- was released by *S. platensis* through cell membrane. Accumulation of OH^- caused alkalinity in pH culture (De-Morais and Costa, 2007). In this study, Production of H_2CO_3 or H^+ was more quickly than production OH^-, so that pH culture had decreasing trend of pH profile (Fig. 4). Liu *et al*. (2008) reported that pH affected the enzymatic activity and electron transport activity of microalgae in the photosynthetic and respiration process, therefore decreasing in pH hampered the growth of *S. platensis*. According to Yang and Gao (2003), culture that contained too much carbon dioxide was toxic for microalgae. Maximum concentration of CO_2 in gas feed was permitted was 5%v/v.

Carbon was main element of microalgae. Content of carbon in microalga was in range 36-58% (Sydney *et al*., 2010). Carbon dioxide was main carbon source that was needed by microalgae for photosynthetic process, but presence of CO_2 had negative effects when it was too much in culture, which were:

- Decreasing the biomass productivity (Watanabe *et al*., 1992)
- Decreasing the pH culture (Falkowski and Raven, 2007)
- Disturbing the photosystem II efficiency (Xu *et al*., 2004). That phenomenon occurred at second 120 min of aeration using biogas. Decreasing of OD_{680} value of culture indicated that an amount of *S. platensis* was death.

S. platensis grew well in range pH of 8-11 Richmond (1988) and Tadros (1988) insisted that concentration of *S. platensis* cell was increasing when pH culture increasing from 8 to 10, then was decreasing when pH culture out of the range. In this study, pH culture was decreasing at first 20 min of cultivation, which pH drop from 9.6 to 7, then pH culture was constant (7-7.1) until at 240th min (Fig. 4). This phenomenon showed that pH culture out of range 8-11 caused *S. platensis* growth inhibition. Kao *et al*. (2012b) stated that pH culture was decreasing as long as 30 min when culture was aerated by using biogas. The lower pH condition of culture, the more dissolved inorganic carbon was in culture. Dissolved inorganic carbon that was too much in culture had toxic characteristic for microalgae.

Effect of cycle-switching operation to growth of *S. platensis* and pH culture: After culture had aerated by using biogas for 240 min, culture aerated by using air until at 600th min (Fig. 3). During cultivation, growth of *S. platensis* and pH culture was increasing gradually (Fig. 3 and 4). This phenomenon was caused by photosynthetic activity, which CO_2 that was dissolved in the culture was utilized by microalgae as carbon source (Kao *et al*., 2012a, b).

Kao *et al*. (2012b) reported that cycle-switching operation, which culture was aerated by using biogas-air simultaneously, affected stable on up-taking of CO_2 by microalgae so microalgae grew well. Cycle-switching operation affected trend of pH culture, which pH culture was being fluctuation during cultivation. pH culture was decreasing when biogas was used as aerator and pH culture was increasing when air was used as aerator (Chiu *et al*., 2011). This result showed the same phenomenon that was reported by the other authors (Kao *et al*., 2012b; Chiu *et al*., 2011) and insisted that cycle-switching operation was better than continuously aeration using biogas or air to grow *S. platensis* (Fig. 2 and 3).

S. platensis had tolerance to CO_2 concentration in range 10-15% (Sydney *et al*., 2010; Kumar *et al*., 2010), that means *S. platensis* could grow well in culture that contained CO_2 concentration in that range. Whereas, in this study, biogas used contained high concentration of CO_2 which was 48.97% sogrowth of *S. Platensis* was hampered when biogas was supplied

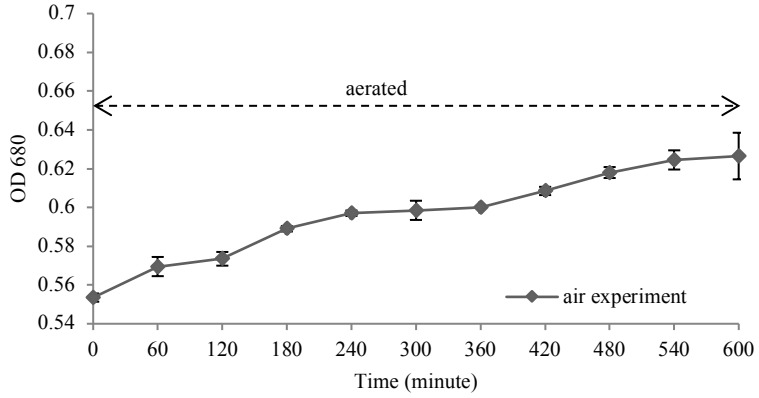


Fig. 2: Growth curve of *S. platensis* in medium aerated using air

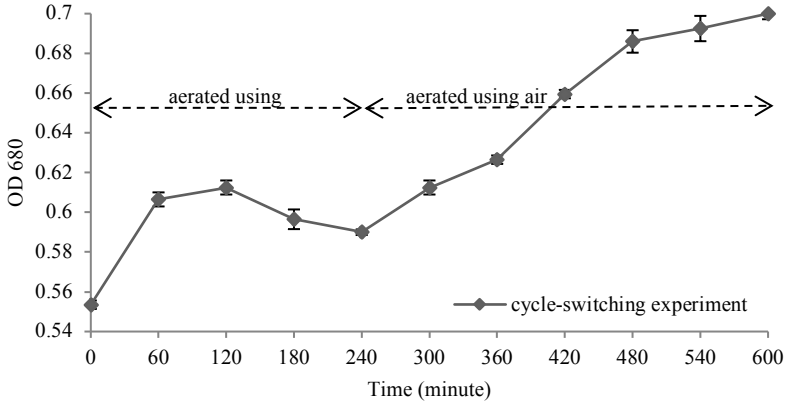


Fig. 3: Growth curve of *S. platensis* in medium aerated using biogas-air (cycle-switching operation)

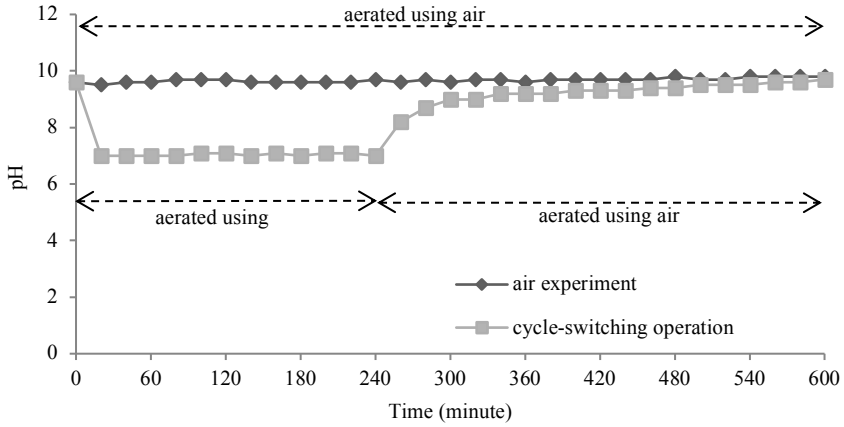


Fig. 4: Profile of pH

continuously into culture. The same results also was reported by Kao *et al*. (2012b), biogas that contained 20±2% CO_2 caused decreasing trend in pH culture and decreasing % CO_2 removal from minute to minute during cultivation. However, the different results was reported by Brown (1996), mutant of *Monoraphidiumminutum* grew well in culture which was supplied by using flue gas contained 13.6% CO_2 and biomass of that was increasing from minute to minute. In addition, growth of *Monoraphidiumminutum* in culture that was aerated by flue gas was faster than that in culture that aerated by air. Condition of pH

Table 3: Growth rate of *Spirulinaplatensis*

Run	$OD_{680\ max}$	T	μ
1	0.6265	600	$0.21*10^{-3}$
2	0.7	600	$0.39*10^{-3}$

Remarks: Run I = aerated by using air; Run 2 = aerated by using biogas-air (cycle-switching operation); t, time at OD 680 max; μ, maximum specific growth rate (/minute)

culture was stable during cultivation. Based on that, we could conclude that each kind of microalgae had the difference of tolerance maximum CO_2 concentration.

Growth rate of *S. platensis* in culture that was aerated by using air and by using biogas-air (cycle-switching operation) can be seen in Table 3. At cycle-switching operation, *S. platensis* could grow well and utilized dissolved CO_2 as inorganic carbon to produce biomass so that growth rate of *S. platensis* in culture of Run 2 was faster than that in cultureof Run 1. Growth rate (μ) on Run 1 and Run 2 were $0.21*10^{-3}$ and $0.39*10^{-3}$/min, respectively.

Kinetic model of *S. platensis* growth: Growth of *S. platensis* was modeled through modified Gompertz equation. Kinetic constant of y, μ and λ was determined by using non-linear regression. Kinetic constants obtained were presented completely in Table 4. By plotting experiment data and simulation of modified Gompertz equation was obtained the graph as shown in Fig. 5.

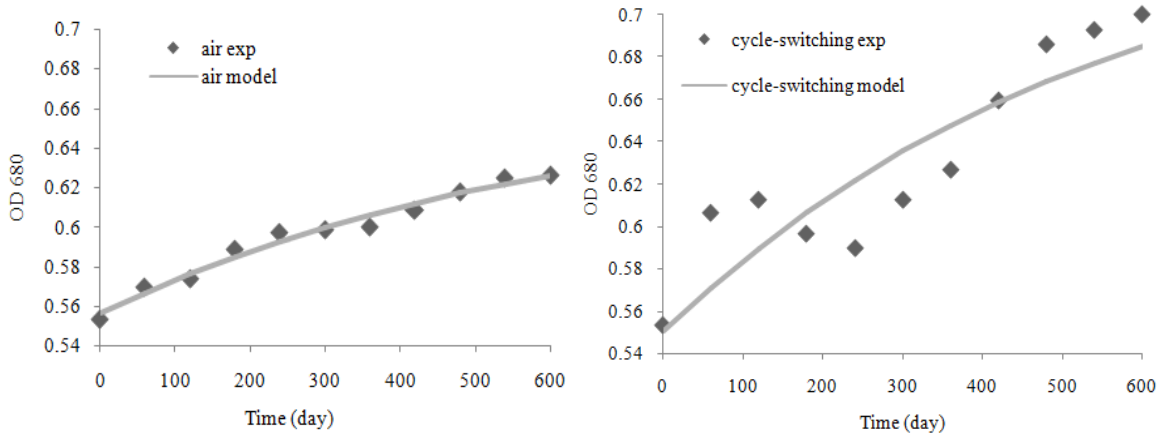
From Table 3, the kinetic constant A value of Run 2 was higher than Run 1, that means value OD_{680} of Run 2 in prediction was more than Run 1. Culture that contained carbon dioxide in appropriate amount was good for microalgae because microalga could do photosynthetic activity to produce biomass (Watanabe *et al*., 1992). However, concentration of carbon dioxide was excess (moreover 10-15%) in culture, it was toxic for *S. platensis* (Sydney *et al*., 2010; Kumar *et al*., 2010). By use of cycle-switching operation (Run 2), concentration dissolved CO_2 in culture could be controlled in other to be not excess, so *S. platensis* activity was not disturbed and also was not lack of carbon dioxide.

Run 2 had kinetic constant of μ that was higher than Run 1, that means growth rate of *S. platensis* in culture of Run 2 was faster than that of Run 1. Culture of Run 1, availability of inorganic carbon was decreasing during cultivation and supplying of carbon dioxide source was not done, so that *S. platensis* did not do photosynthetic and finally death. Meanwhile *S. platensis* in Run 2 could do photosynthesis continuously because supplying of carbon dioxide was done periodically (cycle-switching operation).

Table 4: Kinetic constant of *S. platensis* growth

Run	A	μ	λ	Model	R^2
1	0.663	$0.459*10^{-3}$	-1454.9	$y = 0.663 * exp\left\{-\exp\left[\frac{0.459 * 10^{-3}.e}{0.663}((-1454.9) - t) + 1\right]\right\}$	0.978
2	0.744	$0.588*10^{-3}$	-1024.5	$y = 0.744 * exp\left\{-\exp\left[\frac{0.588 * 10^{-3}.e}{0.744}((-1024.5) - t) + 1\right]\right\}$	0.795

Remarks: Run I = aerated using air; Run 2 = aerated using biogas-air (cycle-switching operation); A, maximum value of OD 680 reached; μ, maximum specific growth rate (/minute); λ, lag time (minutes); R^2, correlation coefficient



(a) (b)

Fig. 5: Comparison of experimental data and modified Gomperz model, (a) in culture aerated by using air, (b) in culture aerated by using cycle-switching operation

CONCLUSION

Biogas could be used as carbon dioxide provider. In this study, biogas used contained 42.37% CH_4; 48.97% CO_2; 1.92% CO and other gases. *S. platensis* could not grow well in culture that was aerated by using biogas continuously. Condition of pH in culture that was aerated by using biogas had decreasing trend so that *S. platensis* was disturbed by this condition. Cycle-switching operation gave the satisfied growth of *S. platensis* which was $0.39*10^{-3}$/min. Whereas growth rate of *S. platensis* in culture that was aerated by using air continuously was $0.21*10^{-3}$/min. Kinetic model of *S. platensis* in culture aerated using by air had kinetic constant of A = 0.663; $\mu = 0.459*10^{-3}$/min; λ = -1454.9 min. Whereas Kinetic model of *S. platensis* in culture aerated by using cycle-switching operation had kinetic constant of A = 0.744; $\mu = 0.588*10^{-3}$/min; λ = -1024.5 min.

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