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Investigation of Interaction Between Light Supply, Carbon and Nitrogen Sources on Growth Rate of *Chlorella vuglaris*

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Abstract: Design of experiments was applied in this study to identify the effects of interaction between environmental conditions on the growth rate of microalgae *Chlorella vulgaris*. *Chlorella vulgaris* were cultivated in flat panel photo-bioreactors with 3 L modified Bold's Basal Medium. A model that can be used to predict the growth rate of *Chlorella vulgaris* with varied bicarbonate concentration (2.5-7.5 g L^{-1}), nitrate concentration (0.5-1.5 g L^{-1}) and light irradiance (2000 LUX-7000 LUX) has been developed. The results showed that the maximum growth rate was obtained at 5 g L^{-1} bicarbonate concentration, 1 g L^{-1} nitrate concentration and light irradiance of 4500 LUX with average value of 0.801 day^{-1}. Besides that, analysis of variance showed that bicarbonate concentration, nitrate concentration and light irradiance individually have significant effect on the growth rate of *Chlorella vulgaris*. Significant interactions were also identified between bicarbonate and nitrate concentrations, as well as nitrate concentration and light irradiance. It is found that with increasing cell population, higher bicarbonate concentration is required to meet the high carbon source demand in culture medium for photosynthesis of microalgae. On the other hand, the photo-inhibition effect of growth rate due to strong light irradiance could be reduced by increasing nitrate concentration in culture medium.

Keywords: *Chlorella vuglaris*, bioreactor, photosynthesis, culture medium

INTRODUCTION

Alternative, renewable and environmental friendly fuel resources are being explored by scientists due to global issue of global warming and fossil fuel depletion (Cole, 2012). One of the alternative energy resources is obtained from the source of human-edible food. However, this is not preferred solution due to global food depletion which is caused by the occurrence of natural disaster. On the other hand, non-edible microalgae with high lipid content can be the candidate of next generation source for bio-fuel production (Phukan *et al*., 2011; Griffiths and Harrison, 2009). Hence, understanding of the microalgae growth plays an important role in improving microalgae cultivation.

Medium composition, especially carbon and nitrogen sources, are the crucial environmental conditions affecting microalgae growth in cultivation (Yeh *et al*., 2010; Chen *et al*., 2010) as carbon and nitrogen sources are used to produce organic compounds and oxygen in the presence of light energy. Considering the low solubility limit of $CO_{2(g)}$ in water (Poling *et al*., 2001), the further increase of $CO_{2(aq)}$ in water become unfavorable. Some studies (Chen *et al*., 2010; Yeh *et al*., 2010; Wijanarko *et al*., 2008) showed that the use of bicarbonate into culture medium provided an alternative solution to substitute the $CO_{2(g)}$ supply and maintain $CO_{2(aq)}$ in culture medium. Besides, bicarbonate is a cheaper carbon source which can be easily manufactured by chemical adsorption of $CO_{2(g)}$ (Trachtenberg and Bao, 2005). Chen *et al*. (2010) and Yeh *et al*. (2010) showed that increasing bicarbonate concentration improved the growth rate of the *Chlorella vulgaris*. On the other hand, nitrate is cheap and is commonly used nitrogen source that affects the growth rate of microalgae. Chen *et al*. (2010) showed that the growth rate of microalgae was enhanced with increasing nitrate concentration.

Another important environmental condition is light irradiance which provides light energy to assist microalgae converting CO_2 into high-energy carbohydrates. Previous study showed that increase in light irradiance resulted in the increment of cell population (Khoeyi *et al*., 2011; Sandnes *et al*., 2005). Although bicarbonate concentration, nitrate concentration and light irradiance are crucial environmental conditions of microalgae cultivation, excessive or extremely weak of environmental conditions inhibited microalgae growth (Carvalho *et al*., 2011; Khoeyi *et al*., 2011; Chen *et al*., 2010).

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Till now, there is lack of study on the effect of interaction between environmental conditions on microalgae growth. The effect of bicarbonate concentration, nitrate concentration and light irradiance on the growth (Chen *et al*., 2010; Riebesell *et al*., 2000; Widjaja *et al*., 2009; Wijanarko *et al*., 2007; Yeh *et al*., 2010) were previously studied by using One Factor At-a-Time (OFAT) which studied one factor at a time instead of all (Czitrom, 1999). This study is aimed at exploring the interaction between environmental conditions which will be investigated by applying Design of Experiment as it can effectively study multiple factors simultaneously compared to OFAT (Czitrom, 1999). Therefore, the growth rate of microalgae can be well understood through the interaction between environmental conditions.

MATERIALS AND METHODS

Experimental setup: *Chlorella vulgaris* strain which was obtained from Commonwealth Scientific and Industrial Research Organization was cultivated in flat panel photo-bioreactor (40 cm length, 10 cm width and 30 cm height) under continuous illumination of fluorescent lamp (6200 K). The culture medium was aerated at a rate of 3.5 L min^{-1} air supply. *Chlorella vulgaris* was cultivated in modified Bold's Basal Medium (Andersen, 2005) which consists of 2.94 mM $NaNO_3$, 0.17 mM $CaCl_2•2H_2O$, 0.304 mM $MgSO_4•7H_2O$, 0.431 mM K_2HPO_4, 1.29 mM KH_2PO_4, 0.428 mM NaCl, 0.171 mM EDTA and 17.9 μM $FeSO_4•7H_2O$.

Growth rate: Daily cell count was performed to generate the growth rate of *Chlorella vulgaris* by using Improved Neubauer Haemocytometer under optical microscope. It is assumed that the cell number increases exponentially during growth phase of the cultivation. The growth rate, μ can be calculated by using:

$$\frac{\ln(N_t/N_0)}{\Delta t} = \frac{\ln N_t - \ln N_0}{(t_t - t_0)}$$

where, N_0 and N_t are the cell count at the beginning and end of a time interval, respectively. Δt is the length of time interval (t_t-t_0).

Statistical analysis: In this study, analysis of Variance (ANOVA) was applied on the growth rate data in order to identify the significant factors and interactions that affect growth rate of *Chlorella vulgaris*. Besides that, a model was developed to predict growth rate of *Chlorella vulgaris*. When ANOVA was applied to the growth rate data, it is assumed that the residuals are normally distributed and the variance of residuals is constant.

Table 1: Growth rate of *Chlorella vulgaris* under factorial design

Bicarbonate (g L^{-1})	Nitrate (g L^{-1})	Light irradiance LUX	Growth rate (day^{-1})
2.5	0.50	7000	0.224
7.5	1.50	2000	0.456
7.5	0.50	7000	0.217
2.5	0.50	2000	0.381
5.0	1.00	4500	0.820
5.0	1.00	4500	0.782
7.5	1.50	7000	0.419
2.5	1.50	2000	0.331
2.5	1.50	7000	0.321
7.5	0.50	2000	0.345

Design of experiments: In this study, the growth rate of *Chlorella vulgaris* was studied by varying the environmental conditions which were bicarbonate concentration (2.5-7.5 g L^{-1}), nitrate concentration (0.5 -1.5 g L^{-1}) and light irradiance (2000 LUX-7000 LUX). The level of light irradiance was measured by using light meter (TENMA 72-6693). Table 1 displays experimental configuration of Factorial Design.

RESULTS AND DISCUSSION

Comparison of growth rate under different environmental conditions: It is observed in Table 1 that maximum growth rate (0.782 and 0.820 day^{-1}) was observed at 5 g L^{-1} bicarbonate concentration, 1 g L^{-1} nitrate concentration and 4500 LUX with average value of 0.801 day^{-1}. Figure 1 displays that the growth rate of *Chlorella vulgaris* grown under different combination of environmental conditions at constant nitrate concentration. As shown in Fig. 1(b), at higher nitrate concentration of 1.5 g L^{-1}, it is observed that growth rate was increased as bicarbonate concentration was increased. With increasing microalgae population, the demand of $CO_{2(aq)}$ became higher (Jeong *et al*., 2003) because more carbon source was required for photosynthesis process in order to allow microalgae to grow faster. This explanation is supported by Riebesell *et al*. (2000) that increase in $CO_{2(aq)}$ can enhance the cell growth.

However, as shown in Fig. 1(a), at nitrate concentration of 0.5 g L^{-1}, there was not much decrease in growth rate as the bicarbonate concentration was increased. This could be due to the fact that when the low nitrate concentration was finished, the microalgae growth was inhibited by nitrate depletion. Thus, the carbon source demand for photosynthesis process was also reduced. It is concluded from Fig. 1 that there was strong interaction between bicarbonate and nitrate concentrations on growth rate of *Chlorella vulgaris*. Another comparison of growth rate is shown in Fig. 2 for different combination of environmental conditions at

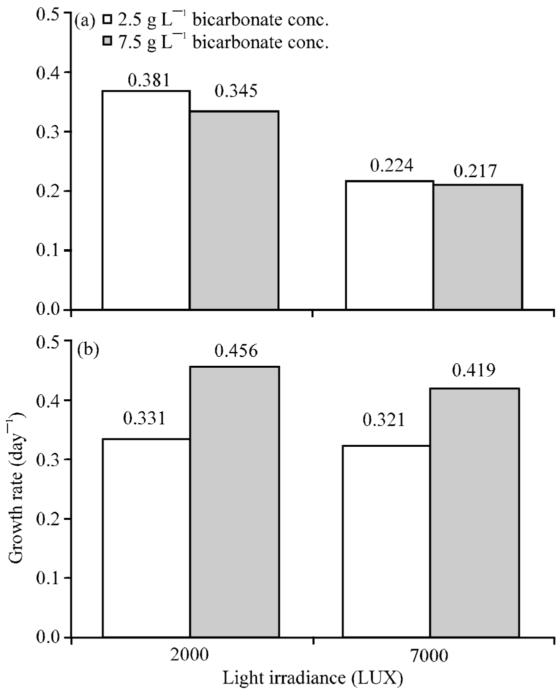

Fig. 1(a-b): Growth rate at constant nitrate concentration (a) 0.5 g L^{-1} and (b) 1.5 g L^{-1}

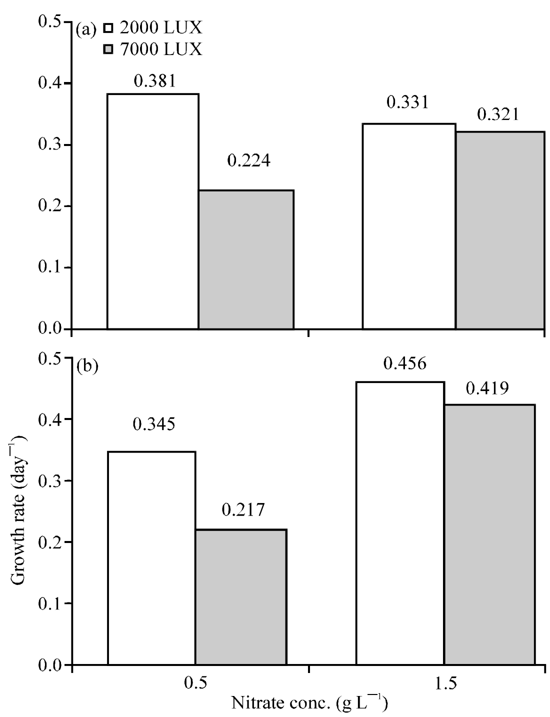

Fig. 2(a-b): Growth rate grown at constant bicarbonate concentration (a) 2.5 g L^{-1} and (b) 7.5 g L^{-1}

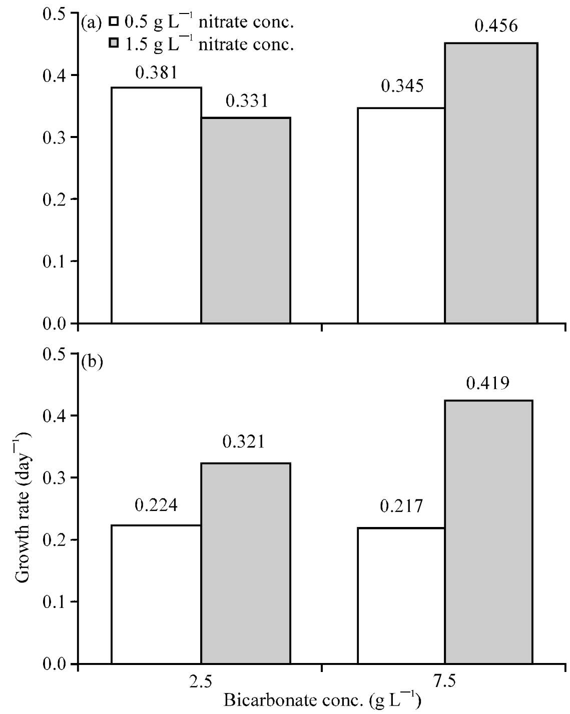

Fig. 3(a-b): Growth rate at constant light irradiance (a) 2000 LUX and (b) 7000 LUX

constant bicarbonate concentration. As displayed in Fig. 2, at 0.5 g L^{-1} nitrate concentration, growth rate was deteriorated by strong light irradiance of 7000 LUX.

However, with increasing light irradiance, no significant change of growth rate was observed at 1.5 g L^{-1} nitrate concentration. These observations showed that the photo-inhibition effect of microalgae growth can be reduced by increasing nitrate concentration to 1.5 g L^{-1}. Therefore, strong interaction was identified between nitrate concentration and light irradiance on growth rate of *Chlorella vulgaris*. On the other hand, as displayed in Fig. 3, the growth rate of *Chlorella vuglaris* was compared under different combination of environmental conditions at constant light irradiance. It was observed that increment of nitrate concentration improved the growth rate of microalgae with increasing bicarbonate concentration. This observation was supported by Chen *et al*. (2010) experiment.

Model analysis: The result of the ANOVA of growth rate is shown in Table 2 where A, B and C represent bicarbonate concentration, nitrate concentration and light irradiance, respectively.

Table 2: Analysis of variance of growth rate

Source	Sum of squares	df	Mean squares	p-value	Significance
Model	0.5100	5	0.1000	0.0015	Significant
A	0.0260	1	0.0260	0.0146	
B	0.1700	1	0.1700	0.0010	
C	0.1500	1	0.1500	0.0011	
AB	0.0640	1	0.0640	0.0040	
BC	0.0970	1	0.0970	0.0022	
Curvature	1.2900	1	1.2900	<0.0001	Significant
Residual	0.0030	3	0.0010		
Lack of fit	0.0019	2	0.0009	0.6133	Not significant
Pure error	0.0011	1	0.0011		
Cor total	1.8000	9			
R-squared	99.41%				

In this study, significance threshold of 0.02 was set. Model p-value of 0.0015 showed that the significant model is built and the significant model terms are A, B, C, AB and BC. Non significance of Lack of Fit implies that the model fit the data well, as supported by p-value of 0.6133. Besides that, R-squared of 99.41% demonstrated that the predicted values from the model are close to experimental values. Therefore, the mathematical model (Eq. 1) for growth rate fitted in terms of actual factors was as follows:

$$\text{Ln (Growth rate)} = -0.51729-0.048991A-0.46567B-1.43455\times10^{-4}+0.071781AB-8.79 \quad (1)$$

Nitrate concentration and light irradiance individually affected the growth rate of *Chlorella vulgaris*, as supported by the average p-value = 0.0010. Besides that, bicarbonate concentration was also significant operation condition affect biomass productivity, as supported by p-value of 0.0146. On the other hand, p-value of bicarbonate and nitrate concentrations (0.0040) showed that the interaction contributes the most in growth rate even though bicarbonate concentration was insignificant environmental condition. Besides that, another significant interaction was also shown by p-value of nitrate concentration and light irradiance (0.0022). These interactions were supported by the previous discussion.

CONCLUSION

In this study, a mathematical model (Eq. 1) was developed to predict the growth rate of *Chlorella vulgaris* and the experimental results showed that the maximum growth rate (0.782 and 0.820 day^{-1}) was obtained at 5 g L^{-1} bicarbonate concentration, 1 g L^{-1} nitrate concentration and 4500 LUX with the average value of 0.801 day^{-1}.

This study showed that nitrate concentration and light irradiance are environmental conditions that significantly affect the growth rate of *Chlorella vulgaris*. Also, it is a new finding that the growth rate was significantly affected by interaction between bicarbonate and nitrate concentrations, as well as interaction between nitrate concentration and light irradiance.

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