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Cell density and light intensity for *Picochlorum sp.*

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Abstract: A *Picochlorum sp.* of *Trebuxiophycean* was previously isolated with the total lipid of 48.6% of its dry weight (DW), including 27.84% of docosahexaenoic acid (DHA). For further fundamental studies and application of the alga such as biomass optimization and lipid production for food and energy, optimal physiological conditions of initial cell density and light intensity are necessary to be determined. The obtained data revealed the best growth of *Picochlorum sp*. was at light intensity of 50µmol photon/m^2/s and cell density of $5x10^6$ cells/ml.

Keywords: Cell, Density, Growth, Light

1. Introduction

Food shortage, climate change, and decrease of fossil fuel availability are global crisis that need alternative sources of nutritional resources and fuel (Chisti 2008, Tran et al. 2014). Microalgal biomass is often rich in products with high nutritional value and pharmaceutical activities and is considered as a good, carbon neutral, renewable energy source (Ordog et al. 2012, Tran et al. 2014). The lipid content and components, as well as proteins, amino acids and vitamins, are greatly different among species/strains, and even within a single strain under various growth conditions (Krienitz and Wirth 2006; Ordog et al. 2012; Ruangsomboon et al. 2013, Tran et al. 2014). An alga *Picochlorum* sp. was previously isolated and determined in our lab as a potential source for food and oil production (Tran et al. 2014), which this research continues to determine optimal conditions of light intensity and cell density for further fundamental studies and application of the alga such as biomass optimization and lipid production for food and energy.

2. Material and Method

2.1. The Alga and Culture Conditions

The alga was previously isolated in Vietnam and maintained in our lab using the low cost modified natural seawater medium 0.5M (MD4) according to Tran *et al.* (2014). The medium contained natural seawater, supplemented with NPK 0.1g/l, $MgSO_4$ 1.86g/l, EDTA 0.00876g/l, $FeCl_3$0.00049g/l, $MnCl_2$ 0.00189g/l, $NaHCO_3$ 50mM, pH = 7.5. The alga was cultivated at three different light intensities (30, 50 and 100µmol photon/m^2/sec) in 50 ml flasks at 25°C to determine the optimal light which was used to grow the alga at three cell densities of $0.5x10^{7,}$ $1x10^7$, $2x10^7$, $4x10^7$ cells/ml. All experiments were done in triplicate and repeated at least twice.

Cell number was counted every three days using a light microscope with 0.1mm deep counting chamber (Neubauer Haemocytometer), and was calculated by the formula:

$$\text{Number of cells/ml} = \text{total cells counted} \times 10^4 \times \text{dilution factor.}$$

Specific growth rate (G: divisions/day) and cell growth productivity (P: cells/ml/day) were determined using equations according to Levasseur et al. (1993):

$$G = \ln(N_t/N_0)/t;\ P = (N_t - N_0)/t$$

Where: N_t and $N_0$are cell density at time t and time 0 respectively.

2.2. Statistical Analysis

All data were calculated with standard error (± ER) and analyzed by one-way ANOVA using SPSS 16.0 software. In all cases, the threshold for significance was set at $p < 0.05$.

3. Result and Discussion

3.1. Optimal Light Intensities

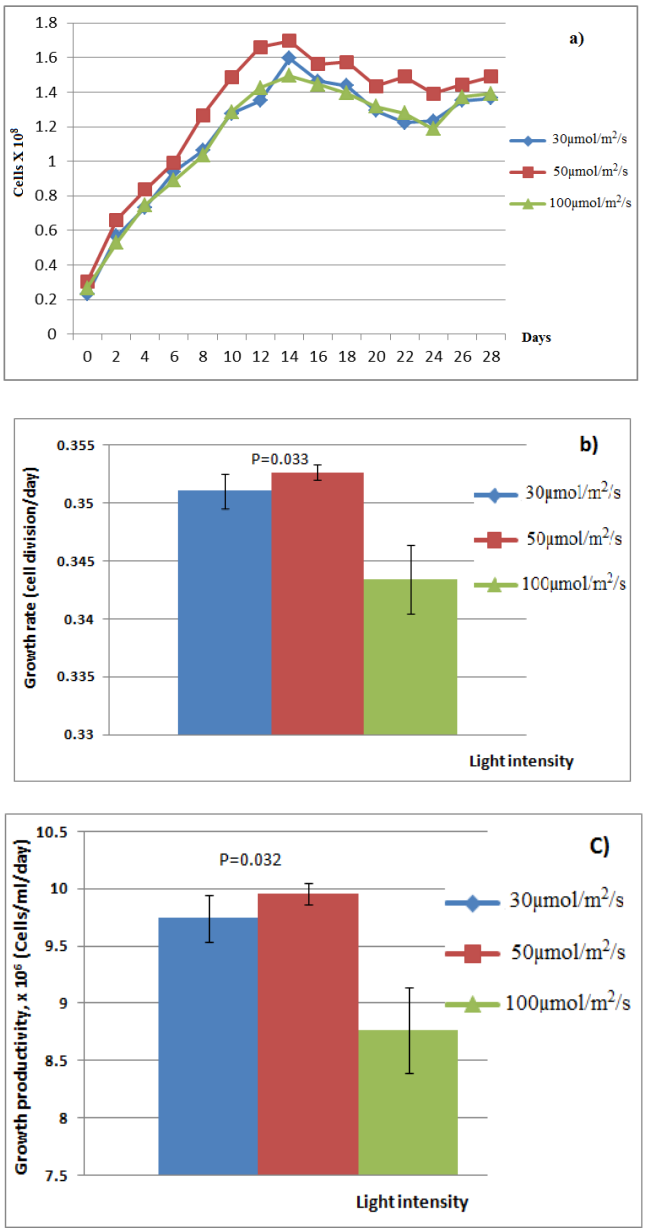

Figure 1. *Growth of Picochlorum sp. under different light intensity of 30, 50, 100 µmoles photons m^{-2} s^{-1}: growth curve (a), growth rate (b) and growth productivity (c) with significant statistic value P*

Data of growth curve, growth rate and productivity (Figure 1) indicates *Picochlorum* sp. grew better at light intensity of 50µmol/m^2/s than at 30 µmol/m^2/s and 100µmol/m^2/s ($p < 0.001$), with respective density of 1.69×10^8 cells/ml, 1.59×10^8 and 1.49×10^8 cells/ml, respectively. There was no difference of growth between light intensity of 30µmol/m^2/s and 100µmol/m^2/s ($p > 0.05$). This indicates both low and high light affect slow growth of *Picochlorum* sp. Light intensity above 50µmol/m^2/s could be applied according to gradual increase of cell density, which needs further tests. The growth began declining after 14 days, but maintained rather stable for at least another 2 weeks. Taken together, the optimal light for *Pichochlorum* was around 50µmol/m^2/s and should be below 100µmol/m^2/s, which is a common preferable light intensity for most marine as well as fresh water microalgae (Vo et al 2014)

3.2. Initial Cell Densities

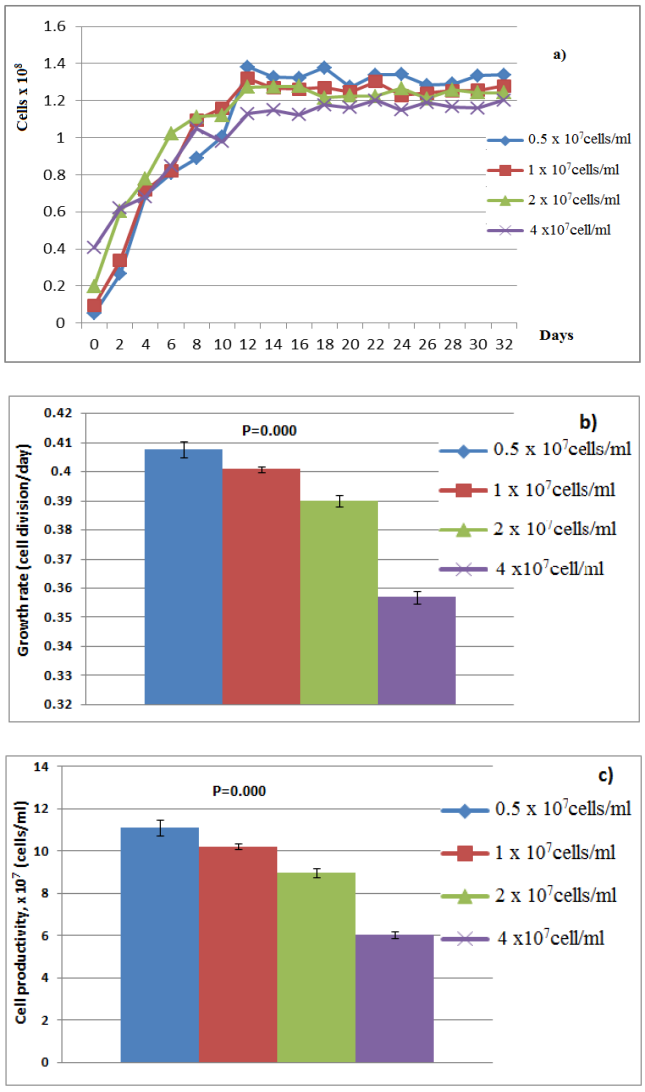

Figure 2. *Growth of Picochlorum sp. from different initial cell densities under optimal light intensity of 50 µmoles photons m^{-2} s^{-1}: growth curve (a), growth rate (b) and growth productivity (c) with significant statistic value P*

Based on the consistent data of growth curve, growth rate and productivity, it revealed that growth of *Picochlorum* from four initial cell densities was significantly different (Figure 2). The initial cell density of 0.5×10^7cells/ml provided the highest growth performance, which reached 1.38×10^8cells/ml after 12 days; whereas the other initial cell densities of 1×10^7, 2×10^7, 4×10^7 cell/ml had lower cell densities of 1.32 $\times 10^8$, 1.27 $\times 10^8$, 1.12 $\times 10^8$ cell/ml, respectively. It is probably that cell density below 0.5×10^7cells/ml could support higher growth. This could be appropriately considered for detailed studies of other physiological conditions at lab scale, but not necessary for large scale cultivation as the cell density needs to be

maintained high for being dominant and resitant to other invading organisms.

Similar to the cell growth data under different light intensities, growth of *Picochlorum* declined after 14 days, and maintained stable growth afterwards. This is an interesting aspect regarding maintaining high biomass with simultaneous accumulation of secondary metabolites such as lipid, antioxidant etc.

4. Conclusion

Light intensity for *Pichochlorum* sp. should be below 100µmol/m^2/s, and the optimal growth was obtained at around 50µmol/m^2/s. The maximum initial cell density should not be above 0.5 x 10^7cell/ml. However, depending on specific study and application, cell density could be applied lower or higher at lab and large scale, respectivey. The data from this research is a base for further fundamental studies and applications of the alga such as biomass optimization and lipid production for food and energy.

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