

Cell density and light intensity for *Picochlorum* sp.

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Abstract: A *Picochlorum* sp. of *Trebuxiophyceae* 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 50 $\mu\text{mol photon/m}^2/\text{s}$ and cell density of 5×10^6 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 100 $\mu\text{mol photon/m}^2/\text{sec}$) in 50 ml flasks at 25°C to determine the optimal light which was used to grow the alga at three cell densities of 0.5×10^7 , 1×10^7 , 2×10^7 , 4×10^7 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 (\pm 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

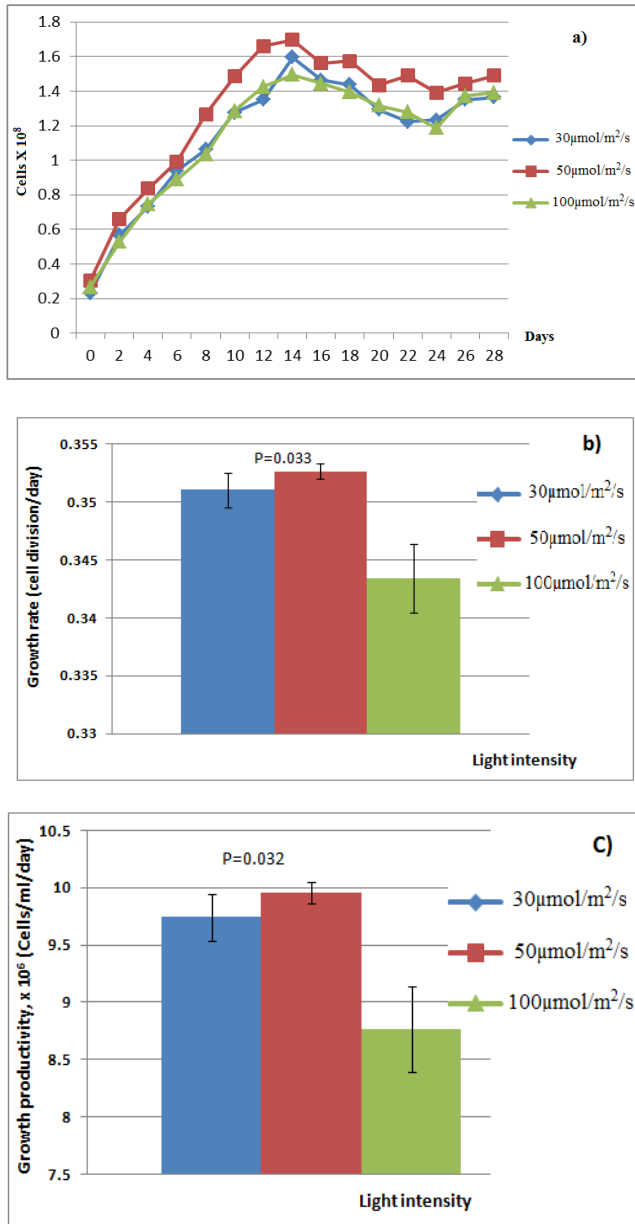


Figure 1. Growth of *Picochlorum* sp. under different light intensity of 30, 50, 100 $\mu\text{mol}/\text{m}^2/\text{s}$: growth curve (a), growth rate (b) and growth productivity (c) with significant statistic value P

Data of growth curve, growth rate and productivity (Figure 1) indicates *Picochlorum* sp. grew better at light intensity of 50 $\mu\text{mol}/\text{m}^2/\text{s}$ than at 30 $\mu\text{mol}/\text{m}^2/\text{s}$ and 100 $\mu\text{mol}/\text{m}^2/\text{s}$ ($p < 0.001$), 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 30 $\mu\text{mol}/\text{m}^2/\text{s}$ and 100 $\mu\text{mol}/\text{m}^2/\text{s}$ ($p > 0.05$). This indicates both low and high light affect slow growth of *Picochlorum* sp. Light intensity above 50 $\mu\text{mol}/\text{m}^2/\text{s}$ 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 *Picochlorum* was around 50 $\mu\text{mol}/\text{m}^2/\text{s}$ and should be below 100 $\mu\text{mol}/\text{m}^2/\text{s}$, 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

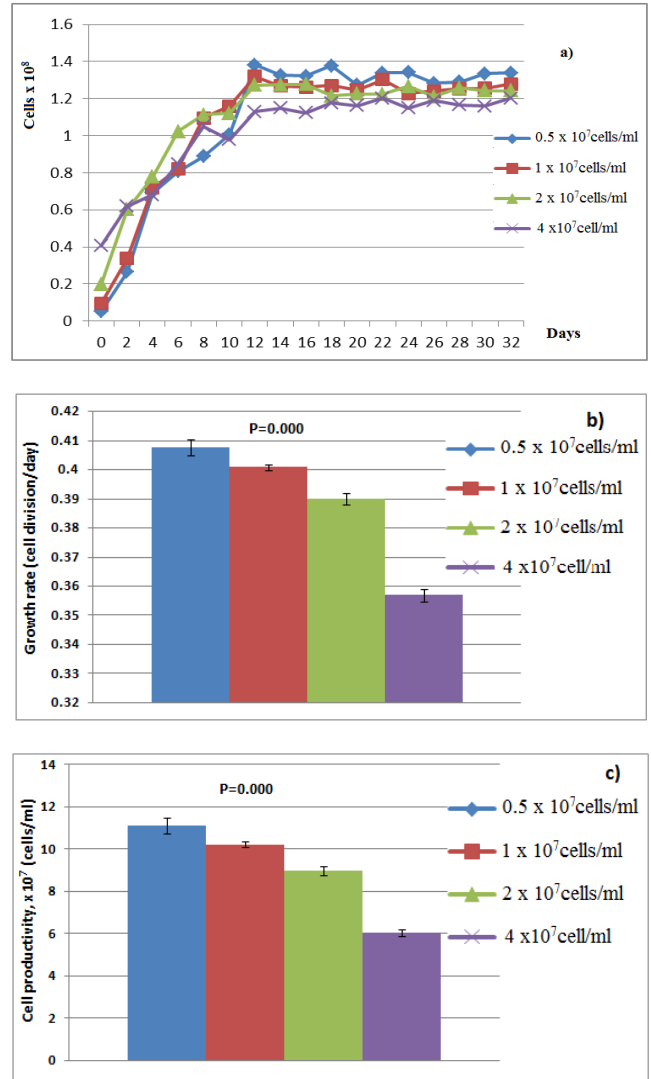


Figure 2. Growth of *Picochlorum* sp. from different initial cell densities under optimal light intensity of 50 $\mu\text{mol}/\text{m}^2/\text{s}$: growth curve (a), growth rate (b) and growth productivity (c) with significant statistic value P

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 $\times 10^7$ cells/ml provided the highest growth performance, which reached 1.38×10^8 cells/ml after 12 days; whereas the other initial cell densities of 1 $\times 10^7$, 2 $\times 10^7$, 4 $\times 10^7$ cell/ml had lower cell densities of 1.32×10^8 , 1.27×10^8 , 1.12×10^8 cell/ml, respectively. It is probably that cell density below 0.5 $\times 10^7$ cells/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 resistant 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 *Picochlorum* sp. should be below $100\mu\text{mol}/\text{m}^2/\text{s}$, and the optimal growth was obtained at around $50\mu\text{mol}/\text{m}^2/\text{s}$. The maximum initial cell density should not be above 0.5×10^7 cell/ml. However, depending on specific study and application, cell density could be applied lower or higher at lab and large scale, respectively. 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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References

- [1] AOAC, 2002(a). Fat (Total, Saturated, Unsaturated, and monounsaturated) in Cereal Products Acid Hydrolysis Capillary Gas Chromatographic Method, AOAC Method 996.01A.
- [2] AOAC, 2002(b). Amino Acids in Feeds Performic Acid Oxidation with Acid Hydrolysis-Sodium Metabisulfite Method. Method 994.12.
- [3] AOAC, 2002(c). Tryptophan in Foods and Food and Feed Ingredients Ion Exchange Chromatographic Method. Method 988.15.
- [4] Aragao, C. L., E.C. Conceicao, M. T. Dinis, and H. J. Fyhn. 2004. Amino acid pools of rotifers and *Artemia* under different conditions: nutritional implications for fish larvae. *Aquaculture* 234: 429–445.
- [5] Arnaud, M. F. 2000. The role of microalgae in aquaculture: situation and trends. *Journal of Applied Phycology* 12: 527–534.
- [6] Bligh, E.G., and W.J. Dyer. 1959. A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemical and Physiology* 37:911–917.
- [7] Borowitzka, M. A. 2013. High-value products from microalgae their development and commercialization. *Journal of Applied Phycology* 25:743–756.
- [8] Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry* 72: 248–254.
- [9] Brown, M. R., C. D. Garland, S. W. Jeffrey, I. D. Jameson, and J. M. Leroi. 1993. The gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis* sp. (clone T.ISO), *Pavlova lutheri* and *Nannochloropsis oculata*. *Journal of Applied Phycology* 5: 285–296.
- [10] Chisti, Y. 2008. Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology*. 26(3): 126–131.
- [11] Chitlaru, E. and U. Pick. 1989. Selection and characterization of *Dunaliella salina* mutants defective in haloadaptation. *Plant Physiology*, 91: 788–794.
- [12] Demirbas, A. 2010. Use of algae as biofuel sources. *Energy Conversion and Management*. 51: 2738–2749.
- [13] Demirbas, F. M. 2011. Biofuels from algae for sustainable development. *Applied Energy* 88: 3473–3480.
- [14] Durmaz, Y., M. Monteiro, N. Bandarra, Ş. Gökpinar, and O. Işık. 2007. The effect of low temperature on fatty acid composition and tocopherols of the red microalga, *Porphyridium cruentum*. *Journal of Applied Phycology* 19:223–227.
- [15] Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package, Version 3.2. *Cladistics* 5: 164–166.
- [16] Francisco, J. L. G., M. Goutx, F. L. Figueroa, and F. X. Niell. 1998. Effects of light intensity, CO₂ and nitrogen supply on lipid class composition of *Dunaliella viridis*. *Journal of Applied Phycology* 10: 135–144.
- [17] Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41:95–98.
- [18] Hanaa, H. A., F. K. El Baz and G. S. El-Baroty. 2004. Production of Lipids Rich in Omega 3 Fatty Acids from the Halotolerant Alga *Dunaliella salina*. *Biotechnology* 3(1): 102–108.
- [19] Harel, M., W. Koven, I. Lein, Y. Bar, P. Behrens, J. Stubblefield, Y. Zohar, and R. A. Place. 2002. Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture* 213:347–362.
- [20] Hu, Q., M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, and A. Darzins. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal*.54:621–639.
- [21] Krientz, L., and M. Wirth. 2006. The high content of polyunsaturated fatty acids in *Nannochloropsis limnetica* (Eustigmatophyceae) and its implication for food web interactions, freshwater. *Limnologia* 36(3): 204–210.
- [22] Lee, S. J., B. D Yoon, and H. M Oh. 1998. Rapid method for the determination of lipid from the green alga *Botryococcus braunii*. *Biotechnology Techniques* 12(7): 553–556.
- [23] Lee, Y. K., H. M. Tan, and C. S. Low. 1989. Effect of salinity of medium on cellular fatty acid composition of marine alga *Porphyridium cruentum* (Rhodophyceae). *Journal of Applied Phycology* 1: 19–23.
- [24] Liang, Y., K. Mai, and S. Sun. 2005. Differences in growth, total lipid content and fatty acid composition among 60 clones of *Cylindrotheca fusiformis*. *Journal of Applied Phycology* 17: 61–65.

- [25] Liu, Z. Y., G. C. Wang, and B. C. Zhou. 2008. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresource Technology* 99: 4717–4722.
- [26] Muradyan, E. A., G. L. Klyachko-Gurvich, N. N. TsogliL, T. V. Sergeyenko, and N. A. Pronina. 2004. Changes in Lipid Metabolism during Adaptation of the *Dunaliella salina* Photosynthetic Apparatus to High CO₂ Concentration. *Russian Journal of Plant Physiology* 51(1): 53–62.
- [27] Nguyen, H. D., V. D. Nguyen, T. H. N. Nguyen, T. T. L. Nguyen, and A. M. Nguyen. 2012. Improvement of amino acid determining method”. *Journal of Science & Technology Development* 14: 27-35.
- [28] Ördög, V., A. S. Wendy, P. Bálint, J. V. Staden, and C. Lovász. 2012. Changes in lipid, protein and pigment concentrations in nitrogen-stressed *Chlorella minutissima* cultures. *Journal of Applied Phycology* 24:907–914.
- [29] Perneta, F., R. Tremblayb, E. Demersc, and M. Roussy. 2003. Variation of lipid class and fatty acid composition of *Chaetoceros muelleri* and *Isochrysis* sp. Grown in a semi-continuous system. *Aquaculture* 221: 393–406.
- [30] Takagi, M., Karseno, and T. Yoshida. 2006. Effect of Salt Concentration on Intracellular Accumulation of Lipids and Triacylglyceride in Marine Microalgae *Dunaliella* Cells. *Journal Of Bioscience And Bioengineering* 101(3): 223–226.
- [31] Renaud, S. M., V. T. Luong, and D. L. Parry. 1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture* 170: 147–159.
- [32] Renaud, S. M., H. C. Zhou, D. L. Parry, V. T. Luong, and K. C. Woo. 1995. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species *Isochrysis* sp. (clone T.ISO). *Journal of Applied Phycology* 7: 595-602.
- [33] Rodolfi, L., G. C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, and M. R. Tredici. 2009. Microalgae for Oil: Strain Selection, Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low-Cost Photobioreactor. *Biotechnology and Bioengineering* 102(1):100-112.
- [34] Ruangsomboon, S., M. Ganmanee, and S. Choochote. 2013. Effects of different nitrogen, phosphorus, and iron concentrations and salinity on lipid production in newly isolated strain of the tropical green microalga, *Scenedesmus dimorphus* KMITL. *Journal of Applied Phycology* 25:867–874.
- [35] Scholz, B., and G. Liebezeit. 2013. Biochemical characterisation and fatty acid profiles of 25 benthic marine diatoms isolated from the Solthörn tidal flat (southern North Sea). *Journal of Applied Phycology* 25:453–465.
- [36] Stein, J. 1973. *Handbook of Phycological methods, Culture methods and growth measurements*. Cambridge University Press.
- [37] Tran D., M. Giordano, C. Louime, N. Tran, T. Vo, D. Nguyen, T. Hoang (2014). An isolated picochlorum sp. for aquaculture, food and biofuel. *North American Journal Of Aquaculture*. DOI: 10.1080/15222055.2014.911226
- [38] Vo T. Vo, D. Tran, 2014. Effects of Salinity and Light on Growth of *Dunaliella* Isolates. *Journal of Applied & Environmental Microbiology*. Vol. 2, No. 5, 208-211. DOI:10.12691/jaem-2-5-2