

Investigating Effects of Sodium Bicarbonate Concentration on Growth of Microalga *Nannochloropsis* sp.

陶氏玄絨、余世宗

E-mail: 381979@mail.dyu.edu.tw

ABSTRACT

Nannochloropsis sp. is a marine microalga that has high oil content on the top of a large biomass production. The purpose of this study was to find the optimum bicarbonate concentration that is suitable for the production of the largest amount of microalgae *Nannochloropsis* sp. The major variable of this study was the feed concentration of carbon source, for which NaHCO_3 was used at concentration ranging from 1 to 30 g- HCO_3^- L⁻¹. Batch type reactors containing the modified Walne medium were operated under growth conditions within favorable ranges. These conditions included: 2.5% NaCl, 50 mg L⁻¹ NO_3^- -N, initial pH of 8.0, and continuous illumination of approximately 12000 lux. Test results showed that *Nannochloropsis* sp. grew to a biomass concentration higher than 0.8 g-dry-wt L⁻¹ under the bicarbonate feed concentration around 14 g- HCO_3^- L⁻¹; biomass productivity reached an observed maximum of 0.56 g-dry-wt L⁻¹ d⁻¹, and the concomitant CO_2 fixation was approximately 85% in a stationary phase attained in about 8 days. Productivity declined from this maximum, most probably due to (1) rise of pH, which was measured to be 9.5 at the stationary phase, from consumption of CO_2 and (2) increased ionic strength in addition to that from NaCl salinity. From the series of growth tests with varying initial feed concentrations of HCO_3^- (Cs), changes in specific growth rate (μ), were determined. Specific growth rate versus HCO_3^- feed concentrations were fitted to the Haldane model as if the feed was inhibitory. The resulting Haldane equation showed that the bicarbonate concentration for optimal production of this algal species was approximately 15 g L⁻¹ with 3 parameters KI, KS and μ .

Keywords : *Nannochloropsis* sp., Sodium bicarbonate, Haldane equation

Table of Contents

中文摘要	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	ix
LIST OF TABLES	xi
Chapter I. INTRODUCTION	1
1.1 Carbon dioxide emission problem	1
1.2 Rationale of this research and objects	1
Chapter II. REVIEW OF LITERATURE	3
2.1 Different ways of carbon dioxide 's mitigation	3
2.2. The roles in nature and promising energy source of <i>Nannochloropsis</i> sp.	5
2.3 Essential nutrients for <i>Nannochloropsis</i> sp. cultivation	7
2.3.1 Macronutrients	8
2.3.2 Micronutrients	11
2.4 Factors affect on the growth of <i>Nannochloropsis</i> sp.	12
2.4.1 Physical factor	13
2.4.2 Chemical factor	15
2.4.3 Operational factors	17
2.5 Photobioreactor	18
2.5.1 Flat – plate photobioreactor	19
2.5.2 Tubular photobioreactor	20
2.5.3 Polyethylene bags	23
2.5.4 Annular columns	25
2.5.5 Stirred – tank photobioreactor	26
Chapter III. MATERIALS AND METHODS	28
3.1 Materials and Instruments	28
3.1.1 Culture medium	28
3.1.2 Microalgae strain	29
3.1.3 Instruments	29
3.1.4 Experiment design	30
3.2 Cultivation system and operation procedure	30
3.3 Method	33
3.3.1 Daily measurement	33
3.3.2 Specific growth rate	34
3.3.3 Biomass productivity	36
3.3.4 Efficiency of carbon fixation	37
3.3.5 Growth kinetic model – Haldane 's equation	40
Chapter IV. RESULTS AND DISCUSSION	44
4.1 Growth characteristic of <i>Nannochloropsis</i> sp.	44
4.2 Variation of pHs in cultures of <i>Nannochloropsis</i> sp.	50
4.3 Variation of dissolved oxygen (DO) concentration in culture medium of <i>Nannochloropsis</i> sp.	53
4.4 Efficiency of carbon fixation by <i>Nannochloropsis</i> sp. at stationary state	54
4.5 Growth kinetic model – Haldane 's equation	55
Chapter V. CONCLUSION	58
REFERENCES	59

REFERENCES

1. Aizawa, K., Miyachi, S., (1986), Carbonic anhydrase and CO_2 concentrating mechanisms in microalgae and cyanobacteria, *FEMS Microbiol. Rev.*, 39, 218-233.
2. APHA, (1998), Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington DC, 1220 p.
3. Aroonwilas, A., Toniwathukul, P., (1997), High-efficiency structured packing for CO_2 separation using 2-amino-2-methyl-1-propanol (AMP), *Separation and Purification Technology*, 12, 67 – 79.
4. Attilio Converti, Alessandro A. Casazza, Erika Y. Ortiz, Patrizia Perego, Marco Del Borghi, (2009). Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing: Process Intensification*, 48, 1146-1151.
5. Audus, H., Oonk, H., (1997), An assessment procedure for chemical utilisation schemes intended to reduce CO_2 emission o atmosphere, *Energy convers. Mgmt*, 38, 409 – 414.
6. Beltrame, P., Beltrame, P.L., Carniti, P., (1980), Use of the Haldane Equation for

steady-state substrate inhibition in biodegradation kinetics, *Biotech. and Bioeng.*, 22, 11, 2405 – 2409. 7. Benemann, J.R., (1992), Microalgae aquaculture feeds, *J. Appl. Phycol.*, 4, 233-45. 8. Best, J. B., (1997), Biodegradation of Naphthalene Using *Pseudomonas putida* (ATCC 17484) in Batch and Chemostat Reactors. Master Thesis, New Jersey Institute of Technology. 9. Biondi, N., Tredici, M., Garefalo, R., (2011), Algae and aquatic biomass for a sustainable production of 2nd generation biofuels, Meger Report, Tanonomy, Biology and Biotechnology. 10. Borowitzka, M. A., (1988), Algal media and sources of algal cultures, *Microalgal Biotechnology*, Borowitzka (Ed.). Cambridge Univ. Press, Cambridge, 456-465. 11. Bold, H. C., Wynne, M. J., (1978), *Introduction to the Algae*. Prentice- Hall, Inc., Englewood Cliffs, New Jersey, 706p. 12. Brand, L. E., Sunda, W. G., Guillard, R. R. L., (1986), Reduction of marine phytoplankton reproduction rates by copper and cadmium, *J. Exp. Mar. Biol. Ecol.*, 96, 225-250. 13. Briassoulis, D., Panagakis, P., Chionidis, M., Tzenos, D., Lalos, A., Tsinos, C., Berberidis, K., Jacobsen, A., (2010), An experimental helical-tubular photobioreactor for continuous production of *Nannochloropsis* sp., *Bioresour. Technol.*, 101, 6768 – 6777. 14. Brown, A.M., (2001), A step-by-step guide to non-linear regression analysis of experimental data using a Microsoft Excel spreadsheet, *J. Comp. Prog. Meth. Biomed.*, 65 (3), 191 – 200. 15. Brown, L.M., (1996), Uptake of Carbon Dioxide from Flue Gas by Microalgae, *J. Energy Conversion Management*, 37, 1363-1367. 16. Chiu, S.Y., Kao, C.Y., Tsai, M.T., Ong, S.C., Chen, C.H., Lin, C. S., (2009), Lipid accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂ aeration, *Bioresour., Technol.*, 100, 833-838. 17. Chu, G.H., Park, J.B., Cheong, M., (2000), Synthesis of dimethyl carbonate from carbon dioxide over polymer supported iodide catalysts, *J. Inorganica Chimica Acta*, 307, 131-133. 18. Colman, B., Rotatore, C., (1995), Photosynthetic inorganic carbon uptake and accumulation in two marine diatoms, *Plant Cell Environ.*, 18, 919-924. 19. Dauta, A., Devaux, J., Piquemal, F., (1990), Growth rate of four freshwater algae in relation to light and temperature. *Hydrobiologia*, 207, 221-226. 20. Darley, W. M. (1982), *Algal biology: A physiological Approach*. Basis Microbiology. Oxford, Blackwell Scientific Publications, 9, 161 – 168. 21. De la Noue, J., Cloutier-Mantha, L., Walsh, P., & Picard, G., (1984), Influence of agitation and aeration modes on biomass production by *Oocystis* sp. grown on wastewaters. *Biomass*, 4, 43-58. 22. De Pauw, N., & Van Vaerenbergh, E., (1983), Microalgal wastewater treatment systems: potentials and limits, *Phytodepuration and the Employment of the Biomass Produced*, Ghetti (Ed.). Centro Ric. Produz. Animali, Reggio Emilia, Italy, 211-287. 23. De Pauw, N., (2004), *Algae culture*, Course, Ghent, Artermia Reference Center, 128p. 24. Devgoswami, Ch.R., Kalita, M.C., Talukdar, J., Bora, R., Sharma, P., (2011), Studies on the growth behavior of *Chlorella*, *Haematococcus* and *Scenedesmus* sp. in culture media with different concentrations of sodium bicarbonate and carbon dioxide gas, *African J. Biotech.*, 10(61), 13128-13138. 25. Feron, P.H.M., Jansen A.E., (1995), Capture of carbon dioxide using membrane gas absorption and reuse in the horticultural industry, *Energy Convers. Mgmt*, 36 (6-9), 411 – 414. 26. Forjan, E., Garbayo, I., Casal, C., Vilchez, C., (2007), Enhancement of carotenoid production in *Nannochloropsis* by phosphate and sulphur limitation, *Communicating current Research and Educational Topics and Trends in Appl. Microbiology*, 356-364. 27. Gouveia, L., Oliveira, A.C., (2009), Microalgae as a raw material for biofuels production, *J. Ind. Microbiol. Biotech.*, 36, 269 – 274. 28. Griffiths, E.W., (2010), *Removal and Utilization of Wastewater Nutrients for Algae Biomass and Biofuels*, Utah State Univ., Logan, Utah, 29. Griffith, M.J., Harrison, S.t.L., (2009), Lipid productivity as a key characteristic for choosing algal species for biodiesel production, *J. Appl. Phycol.*, 21, 493 – 507. 30. Grobbelaar, J. U., (1994), Turbulence in mass algal culture and the role of light/dark fluctuations. *J. Appl. Phycol.*, 6, 331-335. 31. Guillard R. R. L., Ryther J.H., (1962), Studies of marine planktonic diatoms, I. *Cyclotella nana* (Hustedt) and *Detonula confervacea* (Cleve). *Can. J. Microbiol.*, 8, 229-239. 32. Hamasaki, A., Shioji, N., Ikuta, Y., Hukuda, Y., Makita, T., Hirayama, K., Matuzaki, H., Tukamoto, T., and Sasaki, S., (1994), Carbon Dioxide Fixation by Microalgal Photosynthesis Using Actual Flue Gas from a Power Plant, *Appl. Bioch. and Biotech.*, 45-46, 799-809. 33. Healy, F. P., (1973), *Inorganic nutrient uptake and deficiency in algae*, CRC Press, 3, 69-113. 34. Hibberd, D. J., (1981), Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae), *Bot. J. Linn. Soc.*, 2 (2), 93 – 119. 35. Hu, H., Gao, K., (2003), Optimization of growth and fatty acid composition of a unicellular marine picoplankton, *Nannochloropsis* sp., with enriched carbon sources, *Biotech. Lett.*, 25, 421-425. 36. Hu, H., Gao, K., (2006), Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO₂ concentration, *Biotech. Lett.*, 28, 987-992. 37. Hu, Q., Richmond, A., (1994), Optimizing the population density in *Isochrysis galbana* grown outdoors in a glass column photobioreactor. *J. Appl. Phycol.*, 6, 391-396. 38. Huertas, E., Montero, O., Lubian, L.M., (2000), Effects of dissolved inorganic carbon availability on growth, nutrient uptake and chlorophyll fluorescence of two species of marine microalgae, *Aquacult. Eng.*, 22, 181 – 197. 39. Hori, Y., Ito, H., Okano, K., Nagasu, K., Sao, S., (2003), Silver-coated ion exchange membrane electrode applied to electrochemical reduction of carbon dioxide, *J. Electrochimica Acta*, 48, 2651 – 2657. 40. Hoshida, H., Ohira, T., Minematsu, A., Akada, R., Nishizawa, Y., (2005), Accumulation of eicosapentatenoic acid in *Nannochloropsis* sp. in response to elevated CO₂ concentrations, *J. Appl. Phycol.*, 17, 29-34. 41. Jiang, L., Luo, S., Fan, X., Yang, Z., Guo, R., (2011), Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO₂, *Appl. Energy*, 88, 3336 – 3341. 42. Kaplan, D., Richmond, A. E., Dubinsky, Z., & Aaronson, S., (1986), *Algal nutrition*, Handbook of microalgal mass culture, Richmond A. (Ed.), CRC Press, Boca Raton, Florida, USA, 147-198. 43. Knud-Hansen, C. F., (1998), *Pond fertilization: Ecomogical approach and practical application: Pond Dynamics/Aquaculture*, Collaborative Research Support Program, Oregon State University, Corvallis, Oregon, 97331-1641, 125p. 44. Kojima, T., Nagamine, A., Ueno, N., Uemiya, S., (1997), Absorption and fixation of carbon dioxide by rock weathering, *Energy Convers. Mgmt*, 38, 461 – 466. 45. Koleli, F., Balun, D., (2004), Reduction of CO₂ under high pressure and high temperature on Pb-granule electrodes in a fixed-bed reactor in aqueous medium, *Appl. Catalysis A, General*, 274 (1-2), 237 – 242. 46. Kurzbaum E., Kirzhner F., Sela Sh., Zimmels Y., Armon R., (2010), Efficiency of phenol biodegradation by planktonic *Pseudomonas pseudoalcaligenes* (a constructed wetland isolate) vs. root and gravel biofilm, *J. Water Research*, 44, 5021 – 5031. 47. Larsdotter, K., (2006), *Wastewater treatment with microalgae – a literature review*, Environmental

microbiology, School of biotechnology, KTH, Stockholm, 31-38. 48. Lavens, P., Sorgeloos, P., (1996), Manual on the Production and Use of Live Food for Aquaculture, Food and Agriculture Organization of the United Nations, Rome, Italy. 298p. 49. Laws, E. A., Terry, K. L., Wickman, J., & Chalup, M., (1983), A simple algal production system designed to utilize the flashing light effect, *Biotech. and Bioeng.*, 25, 2319-2335. 50. Miron, A.S., Garcia, M.C.C., Gomez, A.C., Camacho, F.G., Grima, E.M., Chisti, Y., (2003) Shear stress tolerance and biochemical characterization of *Phaeodactylum tricornutum* in quasi steady-state continuous culture in outdoor photobioreactors, *Biochem. Eng. J.*, 16, 287 – 297. 51. Mitsuhashi, S., Hosaka, K., Tomonaga, E., Muramatsu, H., (1995), Effects of shear flow on photosynthesis in a dilute suspension of microalgae, *Appl. Microbiol. and Biotech.*, 42, 744-749. 52. Moreno-Garrido I., Blasco J., Gonzalez-Delvalle M., Lubian L.M., (1998), Differences in Copper accumulation by the marine microalga *Nannochloropsis gaditana*, submitted to two different thermal treatments, *Ecotox. Environ. Rest.*, 1 (1), 43-47. 53. Muggli, D. L., Harrison, P. J., (1996), EDTA suppresses the growth of oceanic phytoplankton from the northeast subarctic Pacific, *J. Exp. Mar. Biol. Ecol.*, 205, 221-227. 54. Ohta, K., Hasioto, A., Mizuno, T., (1995), Electrochemical reduction of carbon dioxide by the use of copper tube electrode, *Energy Convers. Mgmt*, 36 (6-9), 625 – 628. 55. Pal D., Khozin-Goldberg I., Cohen Z., Boussiba S., (2011), The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp., *Appl. Microbiol. Biotech.*, 90, 1429 – 1441. 56. Persoone, G. J. M., Verlet H., De Pauw, N., (1980), Air - lift pumps and the effect of mixing on algal growth. *Algae Biomass*, Shelef, S. & Soeder (Eds). Amsterdam: Elsevier/North-Holland Biomedical Press, 505-522. 57. Provasoli, L., Carlucci, A. F., (1974), Vitamins and growth regulators, *Algal Physiology and Biochemistry*, Stewart, W. D. P (Ed.). Oxford - UK: Blackwell Scientific Publ., 741-787. 58. Qi, H., Rorre, G.L., (1995), Photolithotrophic Cultivation of *Laminaria saccharina* Gametophyte Cells in a Stirred-Tank Bioreactor, *Biotech. and Bioeng.*, 45(3), 251 – 260. 59. Recht L., Zarka A., Boussiba S., (2012), Patterns of carbohydrate and fatty acid changes under nitrogen starvation in the microalgae *Haematococcus pluvialis* and *Nannochloropsis* sp., *Appl. Microbiol. Biotechnol.*, 94, 1495 – 1503. 60. Richmond, A., (1996), Efficient utilisation of high irradiance for production of photoautotrophic cell mass: a survey, *J. Appl. Phycol.*, 8, 381-387. 61. Richmond, A., (1987), The challenging confronting industrial microalgae: High photosynthetic efficiency and large-scale reactors *Hydrobiologia*, 151/152, 117-121. 62. Richmond, A., Becker, E. W., (1986), Technological aspects of mass cultivation - a general outline, *CRC Handbook of Microalgal Mass Culture*, Richmond, A. (Ed.). Boca Raton, Florida, USA. CRC Press Inc., 245-263. 63. Richmond, A., Zhang, C.W., (2001), Optimization of a flat plate glass reactor for mass production of *Nannochloropsis* sp. outdoor, *J. Biotech.*, 85, 259 – 269. 64. Rifka Aisyah, (2012, September 12), Effect of pH on microalgae growth, [Online]. (URL <http://www.researchalgae.com/basic/effect-of-ph-on-microalgae-growth.html>). (Accessed 10 October 2012). 65. Rocha, J.M., Gracia, J. E., Henriques, M.H., (2003), Growth aspects of the marine microalga *Nannochloropsis gaditana*, *Biomol. Eng.*, 20 (4-6), 237 – 242. 66. Rodolfi, L., Zittelli, G. C., Bassi, N., Padovani, G.; Biondi, N.; Bonini, G.; Tredici, M. R., (2009), Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, *Biotechnol. Bioeng.*, 102, 100 – 112. 67. Sandnes, J.M., Kallqvist, T., Wenner, D., Gislerod, H.R., (2005), Combined influence of light and temperature on growth rates of *Nannochloropsis oceanica*: linking cellular responses to large-scale biomass production, *J. Appl. Phycol.*, 17, 515 – 525. 68. Shen, Y.M., Duan, W.L., Shi, M., (2004), Chemical fixation of carbon dioxide co-catalyzed by a combination of Schiff bases or Phenols and organic bases, *European J. Organic Chem.*, 14, 3080 – 3089. 69. Sereti, V., (2004), Aquaculture production systems. Master of Science in Aquaculture- Wageningen University, Netherland, 33p. 70. Spolaore, P., et al., (2006), Optimization of *Nannochloropsis oculata* growth using the response surface method, *J. Chem. Technol. Biotech.*, 81, 1049 – 1056. 71. Sudhakar, K., Suresh, S. Premalatha, M., (2011), An overview of CO₂ mitigation using algae cultivation technology, *International J. Chem.*, 3 (3), 110-117. 72. Sukenik, A., Carmeli, Y., (1989), Regulation of fatty acid composition by irradiance level in the Eutigmatophyte *Nannochloropsis* sp., *J. Phycol.*, 25, 686-692. 73. Talbot, P., Thebault, J. M., Dauta, A., (1991), A comparative study and mathematical modeling of temperature, light and growth of three microalgae potentially useful for wastewater treatment, *Water Res.*, 25, 465-472. 74. Tredici, M.R., (2004), Mass Production of Microalgae: Photobioreactors, Richmond, A., (ed.) *Handbook of Microalgal Culture*, Blackwell Science Ltd, Oxford, 1-78?214. 75. Tredici, M.R., Chini Zittelli, G., Rodolfi, L., (2010), Photobioreactors, Flickinger M.C., Anderson S. (eds) *Encyclopedia of Industrial Biotechnology, Bioprocess, Bioseparation, and Cell Technology*. John Wiley & Sons, Inc., Hoboken, NJ, USA, 6, 3-821?3838. 76. Veron, B., Dauget, J. C., Billard, C., (1988), Sterolic biomarkers in marine phytoplankton. II. Free and conjugated sterols of sterols of seven species used in mariculture, *J. Phycol.*, 34, 273 – 279. 77. Wang, B, Li, Y., Wu, N., Lan. C.Q., (2008), CO₂ bio-mitigation using microalgae, *Appl. Microbiol. Biotech.*, 79, 707-718. 78. Zhang, X., Zhao, N., Wei, W., Sun, Y., (2006), Chemical fixation of carbon dioxide to propylene carbonate over amine-functionalized silica catalysts, *Catalysis Today*, 115, 102 – 106. 79. Zittelli, G. C., Rodolfi L., Tredici, M. R., (2003), Mass cultivation of *Nannochloropsis* sp. in annular reactors, *J. Phycol.*, 15, 107 – 114. 80. Zittelli, G. C., Lavista, F., Bastianini, A., Rodolfi, L., Vincenzini, M., Tredici, M. R., (1999), Production of eicosapentaenoic acid by *Nannochloropsis* sp. culture in outdoor tubular photobioreactors, *J. Biotech.*, 70, 299 – 312.