Study on The Production of Phycobiliproteins by Isolated Oscillatoria sp. Wu1

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ABSTRACT

Phycobiliproteins are water-soluble naturally occurring light harvesting pigments commonly present in cyanobacteria and some eukaryotic algae. Phycobiliproteins are covalently attached linear tetrapyrrole chromophoric group called bilins or phycobilins. Moreover, among which the phycocyanin (Phycocyanin) as the main ingredient。This Phycocyanin is a natural pigment, with high nutrients, can be used as food supplements, but also as a natural pigment used in cosmetics。In addition, can also be used in immunoassay, anti-inflammatory and anti-cancer drugs. In this study, cyanobacteria Oscillatoria sp. Wu1 was isolated from seawater around Taiwan, conditions for the production, extraction and purification methods were discussed. The phycocyanin and allophycocyanin content reached 380 mg/g and 103mg/g of dried cell weight in sample from Oscillatoria sp. Wu1 growth conditions: carbon source: 1.0 g-fructose/L, nitrogen source: 0.5 g-urea/L, light intensity: 4300 lux and temperature: 30°C. In addition, the light sources affect the test results found that when the medium containing both carbon / nitrogen source, light sources for Oscillatoria sp. Wu1 in the growth and pigment content of the more obvious. Conversely, when the medium without containing carbon / nitrogen source, light sources of cyanobacteria growth and pigment content of the less obvious. The use of specific wavelengths of light to culture Oscillatoria sp. Wu1 production phycocyanin, places red light (630 nm) light source as training, can achieve the most desired results. In the complex purification process, the use of Molecular gel chromatography to purify. Pure Phycocyanin was finally obtained from Oscillatoria sp. Wu1 with purity ratio (A620/A280) 3.76, further the molecular weight was confirmed by SDS – PAGE.

Keywords : Oscillatoria sp.、Phycobiliprotein、Phycocyanin、Purification

Table of Contents

封面內頁 簽名頁 中文摘要 iii 英文摘要 v 誌謝 vi 目錄 viii 圖目錄 xii 表目錄 xviii 1.前言 1 2.文獻回顧 4 2.1藻膽蛋白(Phycobiliprotein)簡介 4 2.1.1 藻藍蛋白 5 2.1.2 藻紅蛋白 7 2.1.3 異藻藍蛋白 9 2.2生產藻藍蛋白之藻株 9 2.2.1 螺旋藻屬(Spirulina sp.) 10 2.2.2 藍綠菌門其餘藻屬 12 2.3 環境因子對於藍綠菌細胞生長及色素蛋白生成之影響 14 2.3.1 碳源 14 2.3.2 氮源 16 2.3.3 光源 18 2.3.4 溫度 20 2.3.5 pH之影響 21 2.4細胞破壞方法之簡介 22 2.4.1 細胞破壞 22 2.4.2 酵素性破壞(Enzymatic disruption) 23 2.4.3 球磨機(Bead mill) 24 2.4.4 超音波(Sonication) 25 2.4.5 熱裂解(Thermolysis) 26 2.5 藻藍蛋白之萃取與純化 27 3. 材料與方法 32 3.1 實驗器材 32 3.1.1 實驗藥品 32 3.1.2 儀器設備 33 3.1.4 掃描式電子顯微鏡(Scanning Electron Microscope, SEM) 35 3.1.5 傅立葉轉換紅外線光譜儀(Fourier Transform Infrared Spectrometer, FTIR) 36 3.2 藍綠菌來源與篩選 36 3.3 藍綠菌之培養 39 3.3.1保存 39 3.3.2培養 39 3.4 藻體細胞破碎 40 3.5 藍綠菌代謝產物分析 41 3.5.1 還原醣分析 41 3.5.2 總醣分析 42 3.5.3 氨氮分析 43 3.5.4 硝酸根離子分析 45 3.6藍綠菌中色素蛋白萃取及分析方法 46 3.6.1藻膽蛋白萃取及含量測定 46 3.6.2葉綠素a及c萃取及含量測定 47 3.7藻膽蛋白之純化及儲存穩定性分析 48 3.7.1分子膠體管柱層析 48 3.7.2儲存環境溫度之穩定性分析 48 3.7.4 SDS-Polyacrylamide gel eletrophoresis(SDS-PAGE) 48 4. 結果與討論 51 4.1 生產藻藍蛋白之藻株篩選 51 4.2 探討藍綠菌經由不同破碎方法對胞內色素蛋白萃取之影響 54 4.3 不同破碎方法之掃描式電子顯微鏡(SEM)觀察 58 4.4 培養基成分之探討 60 4.4.1 最適氮源探討 60 4.4.2 最適氮源濃度探討 68 4.4.3 最適碳源探討 76 4.4.4 最適碳源濃度探討 86 4.5 環境因子之探討 96 4.5.1 不同培養體積對藻株生產藻藍蛋白之影響 96 4.5.2 探討添加碳/氮源於不同光源波長下對於藻膽色素含量之影響性 105 4.5.3藻株培養於不同溫度下生產藻藍蛋白之影響 131 4.5.4藻株培養於不同pH下生產藻藍蛋白之影響 140 4.6 藻藍蛋白之純化 150 4.7 傅立葉轉換紅外線光譜儀(Fourier Transform Infrared Spectroscopy, FTIR) 154 4.8 藻藍蛋白之儲存穩定性分析 157 5. 結論 160 參考文獻 162 圖目錄 Figure 1-1 Schematic description of this study. 3 Figure 2-1 Structre of phycobilisomes. 5 Figure 2-2 Chemical structure of phycocyanin. 7 Figure 2-3 Chemical structure of phycoerythrin. 8 Figure 2-4 Photosynthetic rate and light intensity diagram. 19 Figure 2-5 Methods of microbial cell disruption. 23 Figure 3-1 Pre-treatment procedures of SEM. 38 Figure 3-2 Schematic description of isolation cyanobacteria. 35 Figure 3-3 The calibration curve of reducing sugar using glucose as a standard agents. 42 Figure 3-4 The calibration curve of total sugar using glucose as a standard agents. 43 Figure 3-5 The calibration curve of NH4+-N. 45 Figure 3-6 The calibration curve of NO3-. 46 Figure 4-1 PC content in the different isolated algae in this study. 53 Figure 4-2 Time course of phycobiliprotein concentration from Oscillatoria sp. Wu1 with homogenization and sonication disrupt methods and different glass size. 56 Figure 4-4 The cell appearance of different fragmentation methods. 59 Figure 4-4 Effect of nitrogen sources on biomass by Oscillatoria sp. Wu1. 63

Figure 4-5 Time courses of pH, glucose, NH4+-N and NO3- concentration from Oscillatoria sp. Wu1 at various nitrogen sources. 64 Figure 4-6 Effect of nitrogen sources on total consumption and consumption rate of glucose by Oscillatoria sp. Wu1. 65 Figure 4-7 Time courses of phycobiliprotein concentration of broth from Oscillatoria sp. Wu1 at various nitrogen sources. 66 Figure 4-8 Effect of nitrogen sources on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1. 67 Figure 4-9 Time courses of pH, glucose, NH4+-N and NO3- concentration by Oscillatoria sp. Wu1 at various urea concentration. 71 Figure 4-10 Effect of urea concentration on total consumption and consumption rate of glucose by Oscillatoria sp. Wu1. 72 Figure 4-11 Effect of urea concentrationon biomass by Oscillatoria sp. Wu1. 73 Figure 4-12 Time courses of phycobiliprotein concentration of broth by Oscillatoria sp. Wu1 at various urea concentration. 74 Figure 4-13 Effect of urea concentrationon on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1. 75 Figure 4-14 Time courses of pH, glucose, NH4+-N and NO3- concentration by Oscillatoria sp. Wu1 at various carbon sources. 80 Figure 4-15 Effect of carbon soures on total consumption and consumption rate of glucose by Oscillatoria sp. Wu1. 81 Figure 4-16 Time courses of phycobiliprotein concentration of broth by Oscillatoria sp. Wu1 at various carbon sources. 82 Figure 4-17 Effect of carbon soures on biomass by Oscillatoria sp. Wu1. 83 Figure 4-18 Effect of carbon sources on chlorophyll yields by Oscillatoria sp. Wu1. 84 Figure 4-19 Effect of carbon sources on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1. 85 Figure 4-20 Effect of fructose concentration on biomass by Oscillatoria sp. Wu1. 90 Figure 4-21 Effect of fructose concentration on total consumption and consumption rate by Oscillatoria sp. Wu1. 91 Figure 4-22 Time courses of f pH, glucose, NH4+-N and NO3- concentration by Oscillatoria sp. Wu1 at various fructose concentration. 92 Figure 4-23 Time courses of phycobiliprotein concentration of broth by Oscillatoria sp. Wu1 at various fructose concentration. 93 Figure 4-24 Effect of fructose concentration on chlorophyll yields by Oscillatoria sp. Wu1. 94 Figure 4-25 Effect of fructose concentration on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1. 95 Figure 4-26 Time courses of pH, glucose, NH4+-N and NO3- concentration by Oscillatoria sp. Wu1 at various medium volume. 99 Figure 4-27 Effect of medium volume on total consumption and consumption rate of glucose by Oscillatoria sp. Wu1. 100 Figure 4-28 Effect of medium volume on biomass by Oscillatoria sp. Wu1. 101 Figure 4-29 Time courses of phycobiliprotein concentration of broth by Oscillatoria sp. Wu1 at various medium volume. 102 Figure 4-30 Effect of medium volume on chlorophyll yields by Oscillatoria sp. Wu1. 103 Figure 4-31 Effect of phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1 at various medium volume. 104 Figure 4-32 Effect of light sources in the medium without carbon and nitrogen on biomass by Oscillatoria sp. Wu1. 114 Figure 4-33 Time courses of pH, exopolysaccharide, NH4+-N and NO3- concentration in the medium without carbon and nitrogen by Oscillatoria sp. Wu1 at various light sources. 115 Figure 4-34 Time courses of phycobiliprotein concentration in the medium without carbon and nitrogen by Oscillatoria sp. Wu1 at various light sources. 116 Figure 4-35 Effect of light sources in the medium without carbon and nitrogen on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1. 117 Figure 4-36 Effect of light sources in the medium without carbon and nitrogen on chlorophyll yields by Oscillatoria sp. Wu1. 118 Figure 4-37 Effect of light sources in the medium containing glucose on biomass by Oscillatoria sp. Wu1. 119 Figure 4-38 Time courses of pH, glucose, NH4+-N and NO3- concentration in the medium containing glucose by Oscillatoria sp. Wu1 at various light source. 120 Figure 4-39 Effect of light sources in the medium containing glucose on total consumption and consumption rate of glucose by Oscillatoria sp. Wu1. 121 Figure 4-40 Time courses of phycobiliprotein concentration in the medium containing glucose by Oscillatoria sp. Wu1 at various light sources. 122 Figure 4-41 Effect of light sources in the medium containing glucose on chlorophyll yields by Oscillatoria sp. Wu1. 123 Figure 4-42 Effect of light sources in the medium containing glucose on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1. 124 Figure 4-43 Effect of light sources in the medium containing glucose and urea on biomass by Oscillatoria sp. Wu1. 125 Figure 4-44 Time courses of pH, exopolysaccharide, NH4+-N and NO3- concentration in the medium containing glucose and urea by Oscillatoria sp. Wu1 at various light source. 126 Figure 4-45 Effect of light sources in the medium containing glucose and urea on total consumption and consumption rate of glucose by Oscillatoria sp. Wu1. 127 Figure 4-46 Time courses of phycobiliprotein concentration in the medium containing glucose and urea by Oscillatoria sp. Wu1 at various light source. 128 Figure 4-47 Effect of light sources in the medium containing glucose and urea on chlorophyll yields by Oscillatoria sp. Wu1. 129 Figure 4-48 Effect of light sources in the medium containing glucose and urea on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1. 130 Figure 4-50 Effect of temperature on biomass by Oscillatoria sp. Wu1. 134 Figure 4-50 Time courses of pH, glucose, NH4+-N and NO3- concentration by Oscillatoria sp. Wu1 at various temperature. 135 Figure 4-51 Effect of temperature on total consumption and consumption rate of glucose by Oscillatoria sp. Wu1. 136 Figure 4-52 Time courses of phycobiliprotein concentration of broth by Oscillatoria sp. Wu1 at various temperature. 137 Figure 4-53 Effect of temperature on chlorophyll yields by Oscillatoria sp. Wu1. 138 Figure 4-54 Effect of temperature on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp. Wu1. 139 Figure 4-55 Time courses of pH, Fructose, NH4+-N and NO3- concentration by Oscillatoria sp. Wu1 at various initial pH. 144 Figure 4-56 Effect of initial pH on total consumption and consumption rate of fructose by Oscillatoria sp. Wu1. 145 Figure 4-57 Effect of initial pH on biomass by Oscillatoria sp. Wu1. 146 Figure 4-58 Time courses of initial pH on phycobiliprotein concentration of broth by Oscillatoria sp. Wu1. 147 Figure 4-59 Effect of initial pH on chlorophyll yields by Oscillatoria sp. Wu1. 148 Figure 4-60 Effect of initial pH on phycocyanin, allophycocyanin, phycoerythrin yields by Oscillatoria sp.

Wu1. 149 Figure 4-61 Column chromatography of phycobiliprotein on a Sephacryl S-200 HR column. 152 Figure 4-62 SDS-PAGE of purifier phycocyanin. 154 Figure 4-63 FTIR spectrum of purification phycocyanin. 156 Figure 4-64 Time courses of storage temperature on phycocyanin concentration. 159 表目錄 Table 2-1 Phycobiliproteins contene of Spirulina. 11 Table 2-2 Phycobiliproteins content of different species. 13 Table 2-3 Phycocyanin;s antioxidant capacity. 31 Table 3-1 Isolated algae medium (IAM). 37 Table 3-2 Culture medium. 40 Table 3-3 The process of broken of algal cells. 41 Table 3-4 SDS-PAGE gel. 50 Table 4-1 Different methods of broken the value of rate constant k. 57 Table 4-2 Summary of the purification of phycocyanin from Oscillatoria sp. Wu1. 153

REFERENCES

1.李東農、郭寶江。1988。光照時間對螺旋藻生長的影響。海洋科學 3: 3-4。 2.林東明。1993。用於食品及化妝品的紅微細藻天然色素。今日經濟一月號:第79-85頁。 3.張成武和殷志敏。1995。藻膽蛋白的特色與利用。中國海洋藥物雜誌 3: 52-53。 4.黃瑞彥。1995。藍點鸚哥魚體內藻膽蛋白之轉變及顏色變化。國立台灣海洋大學食品科學系碩士論文。基隆。 5.蔡心涵。1995。螺旋藻藻藍蛋白對癌激發療法增敏作用的實驗研究。中國海洋藥物雜誌第一期:第15-18頁。 6.鄭俊明。1999。頭髮菜藻紅蛋白異價體性質分析研究。國立台灣大學海洋研究所博士論文。台北。 7.羅曉艷、沉艷、任體輝。2008。水中硝酸根分析方法的改進。化肥工業 35: 58-60。 8.Abalde, J., Betancourt, L., Torres, E., Cid, A. and Barwell, C. 1998. Purification and characterization of phycocyanin from the marine cyanobacterium Synechococcus sp. IO9201. Plant Sci 136: 109-120. 9.Adir, N., Dobrovetsky, Y., Lerner, N. 2001. Structure of c-phycocyanin from thermophilic cyanobacterium Synechococcus vulcanus at 2.5 A: structural implications for thermal stability in phycobilisome assembly. J Mol Biol 313: 71-81. 10.Adir, N., Vainer, R., Lerner, N. 2002. Refined structure of c-phycocyanin from the cyanobacterium Synechococcus vulcanus at 1.6 A: insights into the role of solvent molecules in thermal stability and co-factor structure. Biochim Biophys Acta 1556: 168-174. 11.Arciero, D. M., Dallas, J. L. and Glazer, A. N. 1988. In vitro attachment of bilins to apophycocyanin. II. Determination of the structures of tryptic bilin peptides derived from the phycocyanobilin adduct. J Biol Chem 263: 18350-18357. 12.Ashokkumar P. and Anand N. 2010. Studies on growth and Phycobilin Pigments of the Cyanobacterium Westiellopsis iyengarii. IJBB 6: 315-323. 13.Ayehunie, S.M., Belay, A., Baba, T. W. and Ruprecht, R. M. 1998. Inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis (Arthrospira platensis). JAIDS: Journal of Acquired Immune Deficiency Syndromes 18: 7-12. 14.Beardall, J., Johnston, A. and Raven, J. 1998. Environmental regulation of CO2-concentrating mechanisms in microalgae. Can J Bot 76: 1010-1017. 15.Behrens, P. W. 2005. Photobioreactor and fermentors: the light and the dark sides of the growing algae. In: Andersen, R.A. (Ed.), Algal Culturing Techniques. Elsevier Academic Press, New York, USA, p. 189-204. 16.Belay, A. 2008. Spirulina platensis (Arthrospira): production and quality assurance. In: Gershwin M.E.; Belay A. (Ed.). Spirulina in human nutrition and health. Taylor & Francis, Portland, USA, p. 2-23. 17.Benedetti, S., Rinalducci, S., Benvenuti, F., Francogli, S., Pagliarani, S., Giorgi, L., Micheloni, M., D ’ Amici, G. M., Zolla, L. and Canestrari, F. 2006. Purification and characterization of phycocyanin from the blue-green alga Aphanizomenon flos-aquae. J Chromatogr B 833: 12-18. 18.Benneman, J. R. 1988. Microalgal Biotechnology: Products, Process and Opportunities. Washington: OMEC International Inc vol. 1. 19.Bergman, B., Gallon, J. R., Rai, A. N. and Stal, L. J. 1997. N2 fixation by nonheterocystous cyanobacteria. FEMS Microbiol Rev 19: 139-85. 20.Bezerra, R. P., Matsudo, M. C., Converti, A., Sato, S. and Carvalho, J. C. M. 2008. Influence of ammonium chloride feeding time and light intensity on the cultivation of Spirulina (Arthrospira) platensis. Biotechnol Bioeng. 100: 297-305. 21.Boussiba, S. and Richmond, A. E. 1979. Isolation and characterization of phycocyanins from the blue-green alga Spirulina platensis. Arch Microbiol 120: 155-159. 22.Boussiba, S. and Richmond, A. E. 1980. C-phycocyanin as a storage protein in the blue-green alga Spirulina platensis. Arch Microbiol 125: 143-147. 23.Carlucci, M. J., Scolaro, L. A., Errea, M. I., Matulewicz, M, C. and Damonte, E. B. 1997. Antiviral activity of natural sulphated galactans on herpes virus multiplication in cell culture. Planta Med 63: 429-432. 24.Carmona, R., Vergara, J. J., Lahaye, M. and Niell, F. X. 1998. Light quality affects morphology and polysaccharide yield and composition of Gelidium sesquipedale (rhodophyceae). J Appl Phycol 10: 323-332. 25.Carvalho, J. C. M., Francisco, F. R., Almeida, K. A., Sato, S. and Converti, A. 2004. Cultivation of Arthrospira (Spirulina) platensis (Cyanophyceae) by fed-batch addition of ammonium chloride at exponentially-increasing feeding rate. J Phycol 40: 589-597. 26.Chaneva, G., Furnadzhieva, S., Minkova, K. and Lukavsky, J. 2007. Effect of light and temperature on the cyanobacterium Arthronema africanum - a prospective phycobiliprotein-producing strain. J Appl Phycol 19: 537 – 544. 27.Chen C.H. 1983. The growth and cellular characteristics of spirulina platensis. Master thesis. 28.Chen, F. and Zhang, Y. 1997. High cell density mixotrophic culture of Spirulina platensis on glucose for phycocyanin production using a fed-batch system. Enzyme Microb Technol 20: 221-224. 29.Chen, F., Zhang, Y. and Guo, S. 1996. Growth and phycocyanin formation of Spirulina platensis in photoheterotrophic culture. Biotechnol Lett 18: 603-608. 30.Chen, H. B., Wu, J. Y., Wang, C. F., Fu, C. C., Shieh, C. J., Chen, C. I., Wang, C. Y. and Liu, Y. C. 2010. Modeling on chlorophyll a and phycocyanin production by Spirulina platensis under various light-emitting diodes. Biochem Eng J 53: 52-56. 31.Chisti, Y. and Moo-Young, M. 1986. Disruption of microbial cells for intracellular products. Enzyme Microb Technol 8: 194-204. 32.Ciferri, O. and Tiboni, O. 1985. The biochemistry and industrial potential of Spirulina. Ann Rev Microbiol 39: 503-526. 33.Cohen, Z., 1986. Products from microalgae. In: Richmond, A. (Ed.), Handbook for Microalgal Mass Culture. CRC Press, Boca Raton, FL., USA, p. 421-454. 34.Colla, L. M., Reinehr, C. O., Recichert, C. and Costa, J. A. V. 2007. Production of biomass and nutraceutical compounds by Spirulina platensis under different temperature and nitrogen regimes. Bioresour Technol 98: 1489-1493. 35.Contreras-Martel, C., Matamala, A., Bruna, C., Poo-Caamano, G., Almonacid, D., Figueroa, M., Martinez-Oyanedel, J. and Bunster, M. 2007. The structure at 2 A resolution of

phycocyanin from Gracilaria chilensis and the energy transfer network in a PC – PC complex. Biophys Chem 125: 388-396. 36.Converti, A., Scapazzoni, S., Lodi A. and Carvalho J. C. M. 2006. Ammonium and urea removal by Spirulina platensis. J Ind Microbiol Biotechnol 33: 8-16. 37.Costa, J. A. V., Cozza, K. L., Oliveira, L. and Magagnin, G. 2001. Different nitrogen sources and growth responses of Spirulina platensis in microenvironments. World J Microbiol Biotechnol 17: 43-442. 38.Dakubu, S. 1976. Cell inactivation by ultrasound. Biotechnol and Bioeng 18: 465-471. 39.Danesi, E. D. G., Rangel-Yagui, C. O. and Carvalho, J. C. M. 2002. An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by Spirulina platensis. Biomass Bionerg 23: 261-269. 40.Danesi, E. D. G., Rangel-Yagui, C. O., Sato, S. and de Carvalho, J. C. M. 2011. Growth and content of Spirulina platensis biomass chlorophyll cultivated at different values of light intensity and temperature using different nitrogen sources. Braz J Microbiol 42: 362-373. 41.Del Campo, J. A., Garcia-Gonzalez, M., Guerrero, M.G. 2007. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Appl Microbiol Biotechnol 74: 1163-1174. 42.Der-Vartanian, D., Espardellier, F. J. and Astier, C. 1981. Contributions of respiratory and photosynthetic pathways of a facultative photoautotrophic cyanobacterium, Aphanocapsa 6714. Plant Physiol 68: 974-978. 43.Deshnium, P., Paithoonrangsarid, K., Suphatrakul, A., Meesapyodsuk, D., Tanticharoen, M. and Cheevadhanarak, S. 2000. Temperature-independent and -dependent expression of desaturase genes in filamentous cyanobacterium Spirulina platensis strain C1 (Arthrospira sp. PCC 9438). FEMS Microbiol Lett 184: 207-213 44.Desikachary, T. V. 1959. Cyanophyta. Indian council of Agricultural Research, New Delhi, India, 686. 45.Doke, J. H. 2005. An improved and efficient method for the extraction of phycocyanin from Spirulina sp. Int J Food Eng 1: 2. 46.Doulah, M. S. 1977. Mechanism of disintegration of biological cells in ultrasonic cavitation. Biotechnol and Bioeng 19: 649-660. 47.Engler, C. R. 1985. Disruption of Microbial Cells, in Comprehensive Biotechnology, M. Moo-Young, Editor., Pegamon Press: Oxford, England. 48.Fabregas, J., Garcia, D., Fernandez-Alonso, M., Rocha, A. I., Gomez-Puertas, P., Escribano, J. M., Otero, A. and Coll, J. M. 1999. In vitro inhibition of the replication of haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. Antiviral Res 44: 67-73. 49.Faucher, O., Coupal, B. and Leudy, A. 1997. Utilization seawater-urea as culture medium for spirulina maxima. Can J Microbiol 25: 752-759. 50.Flores, E. and A. Herrero. 1994. Assimilatory nitrogen metabolism and its regulation. In: D. A. Bryant (ed.), The molecular biology of cyanobacteria. p. 487-517. Kluwer Academic Publishers, Dordrecht, The Netherlands. 51.Furuki, T., Maeda, S., Imajo, S., Hiroi, T., Amaya, T., Hirokawa, T., Ito, K. and Nozawa, H. 2003. Rapid and selective extraction of phycocyanin from Spirulina platensis with ultrasonic cell disruption. J Appl Phycol 15: 319-324. 52.Galland-Irmouli, A. V., Pons, L., Lucon, M., Villaume, C., Mrabet, N. T., Gueant, J. L. and Fleurence, J. 2000. One-step purification of R-phycoerythrin from the red macroalga Palmaria palmate using preparative polyacrylamide gel electrophoresis. J Chromatogr B 739: 117-123. 53.Gantt, E. and Lipschultz, C.A. 1973. Energy transfer in phycobilisomes from phycoerythrin to allophycocyanin. Biochim Biophys Acta 292: 858-861. 54.Gantt, E., and Lipschultz, C. A. 1974. Phycobilisomes of Porphyridium cruentum: Pigment analysis. Biochemistry 13: 2960-2966. 55.Gao, K., Li, P., Watanabe, T. and Helbling, E. W. 2008. Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of Arthrospira (Spirulina) platensis (Cyanophyta). J Phycol 44: 777-786. 56.Geciova1, J., Bury, D. and Jelen, P. 2002. Methods for disruption of microbial cells for potential use in the dairy industry-a review. Int Dairy J 12: 541-553. 57.Glazer, A. N. 1982. Phycobilisomes: Structure and Dynamics. Annu Rev Microbiol 36: 173-198. 58.Glazer, A. N. 1984. Phycobilisome A macromolecular complex optimized for light energy transfer. Biochim Biophys Acta 768: 29-51. 59.Glazer, A. N. 1988. Phycobiliproteins. Methods Enzymol 167: 291-303. 60.Glazer, A. N. 1989. Light guides. Directional energy transfer in a photosynthetic antenna. J Biol Chem 264: 1-4. 61.Godinez-Ortega J. L., Snoeijs, P., Robledo, D., Freile-Pelegrin, Y. and Pedersen, M. 2007. Growth and pigment composition in the red alga Halymenia floresii cultured under different light qualities. J Appl Phycol 20: 253-260. 62.Gonzalez, R., Rodriguez, S., Romay, C., Ancheta, O., Gonzalez, A., Armesto, J., Remirez, D. and Merino, N. 1999. Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. Pharmacol Res 39: 55-59. 63.Griffiths, D. J., Thresher, C. L., Street, H. E. 1960. The heterotrophic nutrition of Chlorella vulgaris. Ann Bot 24: 1-11. 64.Grossman, A., Schaefer, M., Chiang, G. and Coller, J. 1993. Environmental effects on the light-harvesting complex of cyanobacteria. J Bacteriol 175: 686-582. 65.Heifetz, P. B., Foster, B., Osmond, C. B., Giles, L. G. and Boynton, J. E. 2000. Effects of acetate on facultative autotrophy in Chlamydomonas reinhardtii assessed by photosynthetic measurements and stable isotope analyses. Plant Physiol 122: 1439-1445. 66.Henrikson R. 1989. In: Henrikson Robert, editor. Earth food Spirulina. Laguna Beach, CA: Ronore Enterprises Inc. 67.Herrera, A., Boussiba, S., Napoleone, V. and Hohlberg, A. 1989. Recovery of C-phycocyanin from the cyanobacterium Spirulina maxima. J Appl Phycol 1: 325-331. 68.Hilditch, C. M., Balding, P., Jenkins, R., Smith, A. J. and Rogers, L. J. 1991. Rphycoerythrin from the macroalga Corallina officinalis (Rhodophyceae) and application of a derived phycofluor probe for detecting sugar-binding sites on cell membranes. J Appl Phycol 3: 345-354. 69.Hirata, T., Tanaka, M., Ooike, M., Tsunomura, T. and Sakaguchi, M. 200. Antioxidant activities of phycocyanobilin prepared from Spirulina platensis. J Appl Phycol 12: 435-439. 70.Holzwarth A. R. 1991. Structure-function relationships and energy transfer in phycobiliprotein antennae. Physiol Plant 83: 518-528. 71.Hoshino, T., Hayashi, T., Hayashi, K., Hamada, J., Lee, J. B. and Sankawa, U. 1998. An antivirally active sulfated polysaccharide from Sargassum horneri (TURNER) C. AGARDH. Biol Pharm Bull 21: 730-734. 72.Kay, R. A. 1991. Macroalgae as food and supplement. Critical Reviews in Food Science Nutrition 30: 555-573. 73.Kitamura, K. 1982. Re-examination of zymolase purification. Biol Chem 446: 963-969. 74.Krimm, S. and Bandekar, J. Vibrational spectroscopy and conformation of peptides, polypeptides and proteins. Adv Protein Chem 38: 181-364. 75.Kula, M. R. and Shutte, H. 1987. Purification of proteins and the disruption of microbial cells. Biotechnol Progr 3: 31-42. 76.Lalucat, J., Imperial, J. and Pares, R. 1984. Utilization of light for the assimilation of organic matter in Chlorella sp. VJ79. Biotechnol Bioeng 26: 677-681. 77.Lee, Y. -K. 2001. Microalgal mass culture systems and methods: their limitation and potential. J Appl Phycol 13, 307-315. 78.Lenton, K. J. and Greenstock, C. L. 1999. Ability of human

plasma to protect against ionising radiation is inversely correlated with age. Mech Ageing Dev 107: 15-20. 79.Lewitus, A. J., Caron, D. A. and Miller, K. R. 1991. Effects of light and glycerol on the organization of the photosynthetic apparatus in the facultative heterotroph Pyrenomonas salina (Cryptophyceae). J Phycol 27:578-587. 80.Li, X., Xu, H. and Wu, Q. 2007. Large-scale biodiesel production from microalga Chlorella protothecoides through heterotrophic cultivation in bioreactors. Biotechnol Bioeng 98: 764-771. 81.Liu, B. H. and Lee, Y. K. 2000. Secondary carotenoids formation by the green alga Chlorococcum sp.. J Appl Phycol 12: 301-307. 82.Carmen, S. S., Teresa, P. N., Roxana, O. R., Jaime, O. L. and Rosa Olivia, C. V. 2004. Extraction and purification of phycocyanin from Calothrix sp. Process Biochem 39: 2047-2052. 83.Madhyastha, H. K. and Vatsala, T. M. 2007. Pigments production in Spirulina fussiformis in different photophysical conditions. Biomol Eng 24: 301-305. 84.Markou, G., and Georgakakis, D. 2011. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. Appl Energy 88: 3389-3401. 85.Marquez, F. J., Saski, K., Kakizono, T., Nishio. N. and Nagai, S. 1993. Growth characteristics of Spirulina platensis in mixotrophic and heterotrophic conditions. J Ferment Bioeng 76: 408-410. 86.Martinez, F. and Orus, M. I. 1991. Interactions between glucose and inorganic carbon metabolism in Chlorella vulgaris strain UAM101. Plant Physiol 95: 1150-1155. 87.Masih, S., Sofi, M. Y. and Singh, S. G. 2011. Growth Performance of Spirulina platensis under Mixotrophic Culture. RJAS 2: 119-121. 88.Masojidek, J., Koblizek, M. and Torzillo, G. 2004. Handbook of microalgal culture: biotechnology and applied phycology, photosynthesis in microalgae. Edited by Amos Richmond, Blackwell Science, 20-39. 89.Middelberg, A. P. J. 1995. Process-scale disruption of microorganisms. Biotechnol adv 13: 491-551. 90.Minkova, K. M., Tchernov, A. A., Tchorbadjieva, M. I., Fournadjieva, S. T., Antova, R. E. and Busheva, MCh. 2003. Purification of C-phycocyanin from Spirulina (Arthrospira) fusiformis. J Biotechnol 102: 55-59. 91.Mishra, S. K., Shrivastav, A. Pancha, I., Jain, D. and Mishra, S. 2010. Effect of preservatives for food grade C-Phycoerythrin, isolated from marine cyanobacteria Pseudanabaena sp.. Int J Biol Macromol 47: 597-602. 92.Monroe, K. and Poore, A. G. B. 2005. Light quantity and quality induce shade-avoiding plasticity in a marine macroalga. J Evol Biol 18: 426-435. 93.Morais, M. and Costa, J. A. V. C. 2007a. Carbon dioxide fixation by Chlorella kessleri, C. Vulgaris, Scenedesmus obliquus and Spirulina sp. cultivated in flasks and vertical tubular photobioreactors. Biotechnol Lett 29: 1349-1352. 94.Morais, M. G. and Costa, J. A. V. C. 2007b. Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. J Biotechnol 129: 439-445. 95.Neppiras, E. A. and Hughes, D. E. 1964. Some experiments on the disintegration of yeast by high intensity ultrasound. Biotechnol and Bioeng 6: 247-270. 96.Nield, J., Rizkallah, P. J., Barber, J., Chayen, N. E. 2003. The 1.45 A threedimensional structure of C-phycocyanin from the thermophilic cyanobacterium Synechococcus elongatus. J Struct Biol 141: 149-155. 97.Niu, J. F., Wang, G. C., Lin, X. Z., Zhou, B. C. 2007. Large-scale recovery of C-phycocyanin from Spirulina platensis using expanded bed adsorption chromatography. J Chromatogr B 850: 267-276. 98.Oesterhelt, C., Schmalzlin, E., Schmitt, J.M. and Lokstein, H. 2007. Regulation of photosynthesis in the unicellular acidophilic red alga Galdieria sulphuraria. Plant J 51: 500 – 511. 99.Oliveira, E. G., Rosa, G. S., Moraes, M. A., Pinto, L. A. A. 2008. Phycocyanin content of Spirulina platensis dried in spouted bed and thin layer. J Food Process Eng 31: 34-50. 100.Ortega-Calvo, J. J., Mazuelos, C., Hermosin, B. and Saiz-Jimenez, C. 1993. Chemical composition of spirulina and eukaryotic algae food products mrketed in spain. J Appl Phycol 5: 425-435. 101.Padayana, A. K., Bhat, V. B., Madyastha, K. M., Rajashankar, K. R., Ramakumar, S. 2001. Crystal structure of a light-harvesting protein C-phycocyanin from Spirulina platensis. Biochem Biophys Res Comm 282: 893-898. 102.Parsons, T. R., Maita, Y. and Lalli, C. M. 1984. A manual of chemical and biological methods for seawater analysis. 1st ed. Pergamon Press, New York , U.S.A. p. 14-17. 103.Patel, A., Mishra, S., Pawar, R. and Ghosh, P. K. 2005. Purification and characterization of C-Phycocyanin from cyanobacterial species of marine and freshwater habitat. Protein Expr Purif 40: 248-255. 104.Patil, G. and Raghavarao, K. S. M. S. 2007. Aqueous two phase extraction for purification of C-phycocyanin. Biochem Eng J 34: 156-164. 105.Patil, G., Chethana, S., Sridevi, A. S., Raghavarao, K. S. M. S. 2006. Method to obtain C-phycocyanin of high purity. J Chromatogr A 1127: 76-81. 106.Perez-Garcia, O., Escalante, F. M. E., de-Bashan, L. E. and Bashan, Y. 2011. Heterotrophic cultures of microalgae: Metabolism and potential products. Water research 45: 11-36. 107.Piorreck, M., Baasch, K. and Pohl, P. 1983. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry 23: 207-216. 108.Pulz, O., Gross, W. 2004. Valuable products from biotechnology microalgae. Appl Microbiol Biotechnol. 65: 635-648. 109.Rangel-Yagui, C. O., Danesi, E. D. G., Carvalho, J. C. M., Sato, S. 2004. Chlorophyll production from Spirulina platensis: cultivation with urea addition by fed-batch process. Biores Technol 92: 133-141. 110.Redlinger, T. and Gantt, E. 1982. A M(r) 95,000 polypeptide in Porphyridium cruentum phycobilisomes and thylakoids: Possible function in linkage of phycobilisomes to thylakoids and in energy transfer. Proc Natl Acad Sci U. S. A. 79: 5542-5546. 111.Reis, A., Mendes, A., Lobo, H., Fernandez, J. and Maggiolly, J. 1998. Production, extraction and purification of phycobiliproteins from Nostoc sp. Biores Technol 66: 181-187. 112.Rhie, G. and Beale, S. I. 1994. Regulation of heme oxygenase activity in Cyanidium caldarium by light, glucose, and phycobilin precursors. J Biol Chem 269: 9620-9626. 113.Richmond, A. 1986a. Cell response to environmental factors. In: Richmond, A. (Ed.), Handbook for Microalgal Mass Culture. CRC Press, Boca Raton, FL., USA, pp. 69-99. 114.Richmond, A. 1986b. Outdoor mass cultures of microalgae. In: Richmond, A. (Eds.), Handbook of Algal Mass Culture. CRC Press, Boca Raton, FL, USA, pp. 285-330. 115.Richmont, A., Karg, S., Boussiba, S. 1982. Effect of bicarbonate and carbon dioxide on the competition between Chlorella vulgaris and Spirulina platensis. Plant Cell Physiol 23:1411-1417. 116.Rimbau, V., Camins, A., Romay, C., Gonzalez, R. and Pallas, M. 1999. Protective effects of C-phycocyanin against kainic acid-induced neuronal damage in rat hippocampus. Neurosci. Lett. 276: 75-78. 117.Rito-Palomares, M., Nunez, L. and Amador, D. 2001. Practical application of aqueous two-phase systems for the development of a prototype process for C-phycocyanin recovery from Spirulina maxima. J Chem Techn Biotechnol 76: 1273-1280. 118.Robert, M. C. 2004. Allophycocyanin and energy transfer. Biochim Biophys Acta 1657: 73-81. 119.Rodrigues, M. S., Ferreira, L. S., Converti, A., Sato, S.

and Carvalho, J. C. M. 2010. Fed-batch cultivation of Arthrospira (Spirulina) platensis: Potassium nitrate and ammonium chloride as simultaneous nitrogen sources. Bioresour Technol 101: 4491-4498. 120.Rodriguez, H., Rivas, J., Guerrero, M. G. and Losada M. 1991. Enhancement of phycobiliprotein production in nitrogen-fixing cyanobacteria. J Bacteriol 20: 263-270. 121.Rogner, M., Nixon, P. J. and Diner, B. A. 1990. Purification and Characterization of Photosystem I and Photosystem II Core Complexes from Wild-type and Phycocyanin-deficient Strains of the Cyanobacterium Synechocystis PCC 6803. J Biol Chem 265: 6189-6196. 122.Romay, C., Gonzalez, R., Ledon, N., Remirez, D. and Rimbau, V. 2003. C-Phycocyanin: a biliprotein with antioxidant, anti-Inflammatory and neuroprotective effects. Curr Protein Pept Sci 4: 207-216. 123.Romay, C., Ledon, N. and Gonzalez, R. 1998. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. Inflamm Res 47: 334-338. 124.Ronda, R.R. and Lele, S.S. 2008. Culture conditions stimulating high γ - linolenic acid accumulation by Spirulina platensis. Braz J Microbiol 39: 693-697. 125.Rossano, R., Ungaro, N., D ' Ambrosio, A., Liuzzi, G. M. and Riccio, P. 2003. Extracting and purifying R-phycoerythrin from Mediterranean red algae Corallina elongate Ellis & Solander. J Biotechnol 101: 289-293. 126.Salasbury, T. 1989. Clarification and extraction: Disruption. In E. L. V. Harris, & S. Angal (Eds.), Protein purification methods: A practical approach. Oxford: IRL Press at Oxford University Press. 127.Santiago-Santos, M. C., Ponce-Noyola, T., Olvera-Ramirez, R., Ortega-Lopez J. and Canizares-Villanueva R. O. 2004. Extaction and purification of phycocyanin from Calothrix sp. Process Biochem 34: 2047-2052. 128.Sarada, R., Pillai, M. G. and Ravishankar, G. A. 1999. Phycocyanin from Spirulina sp: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. Process Biochem 34: 795-801. 129.Sauer, T., Robinson, C. W. and Glick, B. R. F. 1989. Disruption of native and recombinant E. coli in a high pressure homogenizer. Biotechnol Bioeng 33: 1330-1342. 130.Schirmer, T., Bode, W. and Huber, R. 1987. Refined three-dimensional structures of two cyanobacterial C-phycocyanins at 2.1 and 2.5 A resolution: A common principle of phycobilin-protein interaction. J Mol Biol 196: 677-695. 131.Schmidt, R. A., Wiebe, M. G. and Eriksen, N. T. 2005. Heterotrophic high celldensity fed-batch cultures of the phycocyanin producing red alga Galdieria sulphuraria. Biotechnol Bioeng 90: 77-84. 132.Schneegurt, M. A., Sherman, D. M., Sherman, L. A. 1997. Growth, physiology, and ultrastructure of a diazotrophic Cyanothece sp. Strain ATCC 51142 in mixotrophic and chemoheterotrophic cultures. J Phycol 33: 632-642. 133.Shutte, H., Kroner, K. H., Hustedt, H. and Kula, M. R. 1983. Experience with a 20 litre industrial bead mill for the disruption of microorganism. Enzyme Microb Technol 5: 143-148. 134.Silveira, S. T., Burkert, J. F. M., Costa, J. A. V., Burkert, C. A. V. and Kalil S. J. 2007. Optimization of phycocyanin extraction from Spirulina platensis using factorial design. Bioresour Technol 98: 1629-1634. 135.Sloth, J. K., Wiebe, M. G. and Eriksen, N. T. 2006. Accumulation of phycocyanin in heterotrophic and mixotrophic cultures of the acidophilic red alga Galdieria sulphuraria. Enzyme Microb Technol 38: 168-175. 136.Soletto, D., Binaghi, L., Ferrari, L., Lodi, A., Carvalho, J. C. M., Zilli, M. and Converti, A. 2008. Effects of carbon dioxide feeding rate and light intensity on the fed-batch pulse-feeding cultivation of Spirulina platensis in helical photobioreactor. Biochem Eng J 39: 369-375. 137.Soletto, D., Binaghi, L., Lodi, A., Carvalho, J. C. M., Converti, A. 2005. Batch and fedbatch cultivations of Spirulina platensis using ammonium sulphate and urea as nitrogen sources. Aquaculture 243: 217-224. 138.Soni, B., Kalavadia, B., Trivedi, U. and Madamwar, D. 2006. Extraction, purification and characterization of phycocyanin from Oscillatoria quadripunctulata—isolated from the rocky shores of Bet-Dwarka, Gujarat, India. Process Biochem 41: 2017-1023. 139.Soni, B., Trivedi, U. and Madamwar, D. 2008. A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from Phormidium fragile and its characterization for antioxidant property. Bioresour Technol 99: 188-194. 140.Spolaore, P., Joannis-Cassan, C. Duran, E. and Isambert, A. 2006. Commercial applications of microalgae. J Biosci Bioeng 102: 87-96. 141.Stanca, D. and Popovici, E. 1996. Urea as nitrogen source in modified Zarrouk medium. Rev Roum Biol Ser Biol Veg 41: 25-31. 142.Stec, B., Troxler, R. F., Teeter, M. M. 1999. Crystal structure of Cphycocyanin from Cyanidium caldarium provides a new perspective in phycobilisome assembly. Biophys J 76: 2912-2921. 143.Susi, H. 1972. Infrared spectroscopy conformation. Methods Enzymol 22: 455-472. 144.Talarico, L. and Maranzana, G. 2000. Light and adaptive responses in red macroalgae: an overview. J Photochem Photobiol B-Biol 56: 1-11. 145.Tan X., Yao L., Gao Q., Wang W., Qi F. and Lu X. 2011. Photosynthesis driven conversion of carbon dioxide to fatty alcohols and hydrocarbons in cyanobacteria. Metab Eng 13: 169-176. 146.Telford, W., Moss, M., Morseman, J. and Allnutt, T. 2001. Cryptomonad algal phycobiliproteins as fluorochromes for extracellular and intracellular antigen detection by flow cytometry. Cytometry 44:16-23. 147.Tomaselli, L., Margheri, M. C. and Sacchi, A. 1995. Effects of light on pigments and photosynthetic activity in a phycoerythrin-rich strain of Spirulina subsalsa Aquat. Microb. Ecol. 9: 27-31. 148.Troxler, R. F., Ehrhardt, M. M., Brown-Mason, A. S. and Offner, G. D. 1981. Primary structure of phycocyanin from the unicellular rhodophyte Cyanidium caldarium. II. Complete amino acid sequence of the b subunit. J Biol Chem 256: 12176-12184. 149.van Liere, L., de Groot, G. J. and Muur, L. R. 1979. Pigment variation with irradiance in Oscilatoria agardhii Gomont in nitrogen (nitrate)-limited chemostat cultures. FEMS Microbiol Lett 6: 337-340. 150.Venkataraman, L. V., Becker, E. W. 1985. Algal cultivation. In: Venkataraman LV, Becker EW, editors. Biotechnology and utilization of algae. The Indian experience, 12-32. 151.Venugopal, V., Prasanna, R., Sood, A., Jaiswal, P. and Kaushik, B. D. 2006. Stimulation of pigment accumulation in Anabaena azollae strains: effect of light intensity and sugars. Folia Microbiol 51: 50-56. 152.Viskari, P. J. and Colyer, C. L. 2003. Rapid extraction of phycobiliproteins from cultured cyanobacteria samples. Anal Biochem 319: 263-271. 153.Viskari, P. J., Kinkade, C. S. and Colyer, C. L. 2001. Determination of phycobiliproteins by capillary electrophoresis with laser-induced florescence detection. Electrophoresis. 22: 2327-2335. 154.Volkmann, H., Imianovsky, U., Oliveira, J. L. B. and Sant ' Anna, E. S. S. 2008. Cultivation of Arthrospira (Spirulina) platensis in desalinator wastewater and salinated synthetic medium: protein content and aminoacid profile. Braz J Microbiol 39: 98-101. 155.Vonshak, A., Chanawongse, L., Bunnag, B. and Tanticharoen, M. 1996. Light acclimation and photoinhibition in three Spirulina platensis (cyanobacteria) isolates. J Appl Phycol 8: 35-40. 156.Watson, J.D., Hopkins, N.H., Roberts, J.W., Steitz, J.A. and Weiner, A. M. 1987. Molecular Biology of the

Gene. CA: Benjamin/Cummings Publishing Co., Inc., p. 507-507. 157.Zhang X. W., Zhang Y. M., Chen F. 1998. Kinetic models for phycocyanin production by high cell density mixotrophic culture of the microalga Spirulina platensis. J Ind Microbiol 21: 283-288. 158.Zhang X. W., Zhang Y. M., Chen F. 1999. Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of Spirulina platensis. Process Biochem 34: 477-481. 159.Zhang, S. P., Xie, J., Zhang, J. P., Zhao, J. Q. and Jiang, L. J. 1999a. Electron spin resonance studies on photosensitized formation of hydroxyl radical by C-phycocyanin from Spirulina platensis. BBA 1426: 205-211. 160.Zhang, Y. M. and Chen, F. 1999b. A simple method for efficient separation and purification of C-phycocyanin and allophycocyanin from Spirulina platensis. Biotechnol Tech 13: 601-603. 161.Zhu, Y., Chen, X. B., Wang, K. B., Li, Y. X., Bai, K. Z., Kuang, T. Y. and Ji, H. B. 2007. A simple method for extracting C-phycocyanin from Spirulina platensis using Klebsiella pneumonia. Appl Microbiol Biotechnol 74: 244-248. 162.Zucchi, M. R. and Necchi, O. Jr. 2001. Effects of temperature, irradiance and photoperiod on growth and pigment content in some freshwater red algae in culture. Phycol Res 49: 103-14. 163.Brejc, K., Ficner, R., Huber, R. and Steinbacher, S. 1995. Isolation, crystallization, crystal structure analysis and refinement of allophycocyanin from the cyanobacterium Spirulina platensis at 2.3 A resolution. J Mol Biol 249: 424-440. 164.Liu, J. Y., Jiang, T., Zhang, J. P. and Liang, D. C. 1999. Crystal structure of allophycocyanin from red algae Porphyra yezoensis at 2.2-A resolution. J Biol Chem 274: 16945-16952. 165.Jiang, T., Zhang, J., and Liang, D. 1999. Structure and function of chromophores in R-Phycoerythrin at 1.9 A resolution. Proteins 34: 224-231. 166.Reynolds, C. S. 1984: The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge. 167.Garcia, M. C. C., Miron, A. S., Sevilla, J. M. F., Grima, E. M., Camacho, F. G. 2005. Mixotrophic growth of Phaeodactylum tricornutum: Influence of different nitrogen and organic carbon sources on productivity and biomass composition. Process Biochem 40: 297-305. 168.Luning, K. 1992. Day and night kinetics of growth rate in green, brown, and red seaweeds. Journal of Phycology 28: 794-803. 169.Reed, G and Nagodawithana, T. W. 1991. Wine yeast. In: Yeast Technology, Van Nostrand Reinhold, New York, pp. 151-224. 170.Wimpenny, J. W. T. 1967. Breakage of Micro-organisms. Process Biochem 2: 41-44. 171.Mccullough, H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. Clin Chim Acta 17: 297-304. 172.Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350-356. 173. http://www.hashbiotech.com/hashbiotech/ps_cphycocyanin.aspx, 2012/7/2