

Study on Volatile Fatty Acids as a Sole Carbon Source on Lipid Accumulation by Mixotrophic Microalgae

陳琦雯、吳建一

E-mail: 386751@mail.dyu.edu.tw

ABSTRACT

Due to the quick development of human activities and consumption of fossil fuels, the energy crisis and environmental pollution associated problems are a major concern of society. Algae have been proposed as a potential renewable fuel source was compared with the conventional oil crops, microalgae are more attractive as feedstock for biodiesel production, due to their high lipid content and photosynthesis efficiency. The apparent benefits of combining microalgal biodiesel production and wastewater treatment are to minimize the use of freshwater, reduce the cost of nutrient addition for microalgal cultivation and removal organic compounds. Even at optimum conditions, about 60 – 70% of the organic matter remains as residue in the wastewater after acidogenic fermentation of anaerobic biological treatment. In order to produce microalgal lipids by microalgal using volatile fatty acids as a sole carbon source that can be transformed to biodiesel fuel, more microalgal isolates were screened from seawater around Taiwan and then identified according to their 18S rRNA gene sequences and morphological characteristics. *Chlorella pyrenoidosa* G23 that were identified had utilized VFAs potential were selected for further study on their characteristics in growth and lipid producing. Acid-rich effluent generated from acidogenic biomethane production process was evaluated as substrate for lipid synthesis by integrating with mixotrophic cultivation of *C.pyrenoidosa* G23 microalgae. Growth parameters and biochemical composition of the microalga *C.pyrenoidosa* G23 cultivated under different mixotrophic conditions were determined and compared to those obtained from a photoautotrophic control culture. Mixotrophic microalgae showed higher specific growth rate, final biomass concentration and productivities of lipids than microalgae cultivated under photoautotrophic conditions. Moreover, supplementation of the with mixed volatile fatty acids solution (Acetic acid, Propionic acid and Butyric acid) led to a significant improvement in microalgal biomass production and carbohydrate utilization when compared with the culture inorganic culture medium, due to the presence of growth promoting nutrients in mixed volatile fatty acids (VFAs). Mixotrophic cultivation of *C.pyrenoidosa* G23 using the main wastewater biomethane production (WBMP) could be considered a feasible alternative to reduce the costs of microalgal biomass production, since it does not require the addition of expensive carbohydrates to the culture medium.

Keywords : *Chlorella pyrenoidosa* G23、mixed volatile fatty acids、Wastewater Biomethane Production

Table of Contents

封面內頁	簽名頁	中文摘要iii	英文摘要iv	誌謝vi	目錄viii	圖目錄xiii	表目錄xxiii
1. 緒論	1.1 前言	1.2 研究動機與目的	3	2. 文獻回顧	2.1 藻類之介紹	2.2 微藻與廢水	6
2.3 廢水中培養微藻之可行性	6	2.3.1 城市廢水	8	2.3.2 農業廢水	8	2.3.3 工業廢水	9
2.3.4 人工廢水	9	2.4 影響藻類生長以及細胞組成之因子	10	2.4.1 碳源	10	2.4.2 氮源	11
2.4.3 磷源(K ₂ HPO ₄)	12	2.4.4 鹽分(NaCl)	13	2.4.5 光照	14	2.5 藻類生長週期及培養方式	15
2.5.1 自營培養	15	2.5.2 異營性	17	2.5.3 混營性	19	2.6 藻類的培養	21
2.6.1 開放式培養	22	2.6.2 封閉式反應器	24	3. 材料與方法	34	3.1 實驗器材	34
3.1.1 實驗藥品	34	3.1.2 儀器設備	35	3.2 採樣地點之選擇	36	3.3 本土海洋藻類之篩選	37
3.4 微藻鑑定	40	3.5 微藻生長因子與VFAs消耗之探討	45	3.6 利用光學顯微鏡、螢光顯微鏡及電子顯微鏡觀察藻體形態及藻細胞油脂之形成	48	3.6.1 光學顯微鏡之觀察(蘇丹黑染色, Sudan Black Staining)	48
3.6.2 螢光顯微鏡之觀察(尼羅紅染色, Nile Blue A Oxazone)	48	3.6.3 掃描式電子顯微鏡(Scanning Electron Microscope, SEM)	49	3.7 微藻濃度之測定方法	49	3.8 pH&光照強度測量	50
3.9 分析方法	50	3.9.1 尿素分析	50	3.9.2 氮氮分析	51	3.9.3 蛋白質分析	52
3.9.4 葉綠素分析	53	3.9.5 脂肪酸分析	53	3.9.6 VFAs含量分析	56	3.10 微藻生長計算	57
4. 結果與討論	59	4.1 海水微藻篩選	59	4.1.1 藻株在WBMP/WBHP培養之初步篩選	59	4.1.2 藻株在VFAs培養基培養下之初步篩選	60
4.1.2.1 VFAs培養基培養中添加蔗糖與海水素對微藻之篩選	64	4.2 在VFAs培養下探討有曝氣對微藻生長及其油脂含產量之影響	68	4.3 探討利用稀釋後的WBMP/WBHP處理後出流水與VFAs培養基培養中添加氮源培養對微藻生長及VFAs消耗之影響	73	4.3.1 稀釋後的WBMP/WBHP處理後出流水	73
4.3.2 VFAs培養基	75	4.4 在曝氣條件下VFAs培養基添加尿素對微藻生長及VFAs消耗之影響	88	4.5 在VFAs培養基培養條件下不同氮源種類對微藻生長及VFAs消耗之影響	95	4.6 在VFAs培養基培養條件下不同氮源濃度對微藻生長及VFAs消耗之影響	107
4.7 探討WBMP放流水在不同透光度下對微藻生長及VFAs消耗之影響	115	4.8 藻株之鑑定與外型	124	4.9 探討不同磷酸氫二鉀(K ₂ HPO ₄)濃度對微藻 <i>Chlorella pyrenoidosa</i> G23生長及VFA消耗率之影響	127	4.10 探討不同NaCl濃度對微藻 <i>Chlorella pyrenoidosa</i> G23生長以及VFA消耗率	133
4.11 探討在VFAs培養下不同曝氣量對微藻 <i>Chlorella pyrenoidosa</i> G23生長及VFAs消耗率之影響	139	4.12 探討混和脂肪酸(醋酸、丙酸和丁酸)含量對微藻 <i>Chlorella pyrenoidosa</i> G23生長及VFAs消耗之影響	145				

4.13 探討在不同光週期下對微藻 *Chlorella pyrenoidosa* G23 生長及 VFAs 消耗之影響 153

4.14 探討不同光源對微藻 *Chlorella pyrenoidosa* G23 生長及 VFAs 消耗之影響 161

4.15 探討在曝氣條件下未滅菌 WBMP 出流水對微藻 *Chlorella pyrenoidosa* G23 生長及 VFAs 消耗率 169

5. 結論 177

參考文獻 179

圖目錄

Figure 1.1 Schematic of this study procedure.

4 Figure 2.1 Heterotrophic metabolism in microalgae.

21 Figure 2.2 Schematics of a raceway pond .

24 Figure 2.3 Photograph and schematics of Vertical tubular reactor.

26 Figure 2.4 Photograph and schematics of bubble column reactor.

27 Figure 2.5 Different types of Airlift reactor.

28 Figure 2.6 Front and side view of the flat panel photobioreactor.

30 Figure 2.7 Working of a horizontal tubular photobioreactor.

31 Figure 2.8 Helical type photobioreactor.

33 Figure 3.1 Collection sites of algal-like microorganism strains established in this study.

38 Figure 3.2 The algae isolation equipment in this study.

40 Figure 3.3 Map of FAMEs.

56 Figure 3.4 Map of VFAs.

57 Figure 4.1 Growth of various isolated microalgae in WBMP after 7 days.

62 Figure 4.2 Growth of various isolated microalgae in WBHP after 7 days.

63 Figure 4.3 Growth of various isolated microalgae in VFAs medium after 7 days (Sterilization).

64 Figure 4.4 Growth of various isolated microalgae in medium with/without containing marine sale (20 g/L) or sucrose (1 g/L) after 7 days.

65 Figure 4.5 Microscopic observation of various isolated microalgae growth with/without containing marine sale (20 g/L) or sucrose (1 g/L) after 7 days.

66 Figure 4.6 Initial screening of microalgae at various isolated microalgae growth with/without containing marine sale (20 g/L) or sucrose (1 g/L) after 7 days.

67 Figure 4.7 Total FAMEs content and growth of various isolated microalgae in VFAs medium without containing marine sale (20 g/L) or sucrose (1 g/L) after 7 days.

70 Figure 4.8 Percentages of individual fatty acid methyl ester on the total FAMEs (%) in VFAs medium without containing marine sale (20 g/L) or sucrose (1 g/L) after 7 days.

71 Figure 4.9 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMEs (%) in VFAs medium without containing marine sale (20 g/L) or sucrose (1 g/L) after 7 days.

72 Figure 4.10 Growth of various isolated microalgae in medium without containing marine sale (20 g/L) or sucrose (1 g/L) after 7 days.

78 Figure 4. 11 Total FAMEs content at eight different microalgae culture in different medium after 7 day culture.

80 Figure 4. 12 Percentages of individual fatty acid methyl ester on the total FAMEs (%) in eight different microalgae culture in different medium after 7 day culture.

81 Figure 4. 13 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMEs (%) in eight different microalgae culture in different medium after 7 day culture.

82 Figure 4. 14 Growth of various isolated microalgae in various nitrogen medium after 7 days.

83 Figure 4. 15 Total FAMEs content at eight different microalgae culture in various nitrogen medium after 7 day culture.

85 Figure 4. 16 Percentages of individual fatty acid methyl ester on the total FAMEs (%) in eight different microalgae culture in various nitrogen medium after 7 day culture.

86 Figure 4. 17 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMEs (%) in eight different microalgae culture in various nitrogen medium after 7 day culture.

87 Figure 4. 18 The time course of dried cell weight of five microalgae at without supplement urea as a nitrogen sources after 7 day culture.

90 Figure 4. 19 Percentages of individual fatty acid methyl ester on the total FAMEs (%) of five microalgae at without supplement urea as a nitrogen sources after 7 day culture.

93 Figure 4. 20 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMEs (%) of five microalgae at without supplement urea as a nitrogen sources after 7 day culture.

94 Figure 4. 21 The time course of growth of five microalgae at various nitrogen sources after 7 day culture.

99 Figure 4. 22 The VFAs consumption of five microalgae at various nitrogen sources after 7 day culture.

100 Figure 4. 23 Percentages of individual fatty acid methyl ester on the total FAMEs (%) in five microalgae at various nitrogen sources after 7 day culture.

104 Figure 4. 24 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMEs (%) in five microalgae at various nitrogen sources after 7 day culture.

105 Figure 4. 25 Scanning electron micrographs of five microalgae (X10000).

106 Figure 4. 26 The time course of growth of five microalgae at various nitrogen (Urea & NH₄NO₃) concentration after 7 day culture.

110 Figure 4. 27 The VFAs consumption of five microalgae at various nitrogen (Urea & NH₄NO₃) concentration after 7 day culture.

111 Figure 4. 28 Total FAMEs content and growth of G11, G23 and RO microalgae at various nitrogen (Urea & NH₄NO₃) concentration after 7 day culture.

112 Figure 4. 29 Percentages of individual fatty acid methyl ester on the total FAMEs (%) in five microalgae at various nitrogen (Urea & NH₄NO₃) concentration after 7 day culture.

113 Figure 4. 30 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMEs (%) in five microalgae at various nitrogen (Urea & NH₄NO₃) concentration after 7 day culture.

114 Figure 4. 31 Under the conditions of different light intensity (OD680).

118 Figure 4. 32 The time course of growth of three microalgae at WBMP waste concentration after 7 day culture.

119 Figure 4. 33 The VFAs consumption of G11, G23 and RO microalgae at WBMP after 7 day culture.

120 Figure 4.34 Total FAMEs content and growth of G11, G23 and RO microalgae at WBMP after 7 day culture.

121 Figure 4. 35 Percentages of individual fatty acid methyl ester on the total FAMEs (%) in G11, G23 and RO microalgae at WBMP after 7 day culture.

122 Figure 4. 36 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMEs (%) in G11, G23 and RO microalgae at WBMP after 7 day culture.

123 Figure 4. 37 Optical Microscope of microalgae *Chlorella pyrenoidosa* G23.

125 Figure 4. 38 Maximum likelihood phylogenetic tree of 18S rRNA gene sequences from microalgae used in this study.

125 Figure 4. 39 The time course of growth of in *Chlorella pyrenoidosa* G23 microalgae at K₂HPO₄ concentration in medium after 7 day .

128 Figure 4. 40 The VFAs consumption of *Chlorella pyrenoidosa* G23 microalgae at K₂HPO₄ concentration after 7 day culture.

129 Figure 4. 41 Total FAMEs content and growth of *Chlorella pyrenoidosa* G23

microalgae at K₂HPO₄ concentration in medium after 7 day.130 Figure 4. 42 Percentages of individual fatty acid methyl ester & saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMES (%) in Chlorella pyrenoidosa G23 microalgae at K₂HPO₄ concentration in medium after 7 day.131 Figure 4. 43 The time course of growth of in Chlorella pyrenoidosa G23 microalgae at NaCl concentration in medium after 7 day.134 Figure 4. 44 The VFAs consumption of Chlorella pyrenoidosa G23 microalgae at NaCl concentration after 7 day culture.135 Figure 4. 45 Total FAMES content and growth of Chlorella pyrenoidosa G23 microalgae at NaCl concentration in medium after 7 day .136 Figure 4. 46 Percentages of individual fatty acid methyl ester & saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMES (%) in Chlorella pyrenoidosa G23 microalgae at NaCl concentration in medium after 7 day .137 Figure 4. 47 The time course of growth of in Chlorella pyrenoidosa G23 microalgae at different aeration rate (0.03 % CO₂) in medium after 7 day . 140 Figure 4. 48 The VFAs consumption of Chlorella pyrenoidosa G23 microalgae at different aeration rate (0.03% CO₂) after 7 day culture. 141 Figure 4.49 Total FAMES content and growth of Chlorella pyrenoidosa G23 microalgae at different aeration rate (0.03% CO₂) in medium after 7 day.142 Figure 4. 50 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition & individual fatty acid methyl ester on the total FAMES (%) in Chlorella pyrenoidosa G23 microalgae at different aeration rate (0.03% CO₂) in medium after 7 day .143 Figure 4. 51 The time course of dried cell weight and nutrients consumption of Chlorella pyrenoidosa G23 microalge at different VFAs content after 7 day culture.148 Figure 4. 52 The intracellular protein, chlorophyll a, chlorophyll b and chlorophyll c, carotenoid content of Chlorella pyrenoidosa G23 microalge at different VFAs content after 7 day culture.149 Figure 4. 53 Total FAMES content and growth of Chlorella pyrenoidosa G23 microalge at different VFAs content after 7 day culture.150 Figure 4. 54 The percentages of individual fatty acid methyl ester & saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMES (%) in Chlorella pyrenoidosa G23 microalage at differentVFAs ratios after 7 day culture.151 Figure 4. 55 The time course of dried cell weight and nutrients consumption of Chlorella pyrenoidosa G23 microalge at various light/dark cycle after 7 day culture.155 Figure 4. 56 The VFAs consumption of Chlorella pyrenoidosa G23 microalgae at varios light/dark cycle after 7 day culture.156 Figure 4. 57The intracellular protein, chlorophyll a, chlorophyll b and chlorophyll c, carotenoid content of Chlorella pyrenoidosa G23 microalge at varios light/dark cycle after 7 day culture.157 Figure 4. 58Total FAMES content and growth of Chlorella pyrenoidosa G23 microalge at various light/dark cycle after 7 day culture.158 Figure 4.59 The percentages of individual fatty acid methyl ester &saturated fatty acid,unsaturated fatty acid and unknow fatty acid composition on the total FAMES (%) in Chlorella pyrenoidosa G23 microalge at various light/dark cycle after 7 day culture.159 Figure 4. 60 The time course of dried cell weight and nutrients consumption of Chlorella pyrenoidosa G23 microalge at various light sources after 7 day culture.163 Figure 4. 61The VFAs consumption of Chlorella pyrenoidosa G23 microalgae at varios light sources after 7 day culture.164 Figure 4. 62 The intracellular protein, chlorophyll a, chlorophyll b and chlorophyll c, carotenoid content of Chlorella pyrenoidosa G23 microalge at varios light sources after 7 day culture.165 Figure 4. 63 Total FAMES content and growth of Chlorella pyrenoidosa G23 microalge at various light souces after 7 day culture.166 Figure 4. 64 The percentages of individual fatty acid methyl ester &saturated fatty acid,unsaturated fatty acid and unknow fatty acid composition on the total FAMES (%) in Chlorella pyrenoidosa G23 microalge at various light souces after 7 day culture.167 Figure 4. 65 The time course of dried cell weight of in Chlorella pyrenoidosa G23 microalge at the conditions of Sterilization and Unsterilization after 7 day culture.170 Figure 4. 66 The VFAs consumption of Chlorella pyrenoidosa G23 microalgae at the conditions of Sterilization and Unsterilization after 7 day culture.171 Figure 4. 67 Total FAMES content and growth of Chlorella pyrenoidosa G23 microalge at the conditions of Sterilization and Unsterilization after 7 day culture.172 Figure 4. 68 The intracellular protein, chlorophyll a, chlorophyll b and chlorophyll c, carotenoid content of Chlorella pyrenoidosa G23 microalge at the conditions of Sterilization and Unsterilization after 7 day culture.173 Figure 4. 69 Percentages of individual fatty acid methyl ester on the total FAMES (%) in Chlorella pyrenoidosa G23 microalge at the conditions of Sterilization and Unsterilization after 7 day culture.174 Figure 4. 70 The percentage of saturated fatty acid, unsaturated fatty acid and unknow fatty acid composition on the total FAMES (%) in Chlorella pyrenoidosa G23 microalgeat the conditions of Sterilization and Unsterilizationafter 7 day culture.175 表目錄 Table 4. 1 At present screening of fifty microalgae.61 Table 4. 2 The VFAs consumption of eight different microalgae culture in different medium after 7 day culture.79 Table 4. 3 The VFAs consumption of eight different microalgae culture in various nitrogen medium after 7 day culture.84 Table 4. 4 The VFAs consumption of five microalgae at without supplement urea as a nitrogen sources after 7 day culture.91 Table 4. 5 Total FAMES content and growth of five microalgae at without supplement urea as a nitrogen sources after 7 day culture.92 Table 4.6 Total FAMES content and growth of five microalgae at various nitrogen sources after 7 day culture.101 Table 4.7 Experimental variation evaluated.147

REFERENCES

1.林良平。1991。小球藻之混營生長及微藻在生態生產上所扮演的角色。中華生質能源學會會誌。10: 89-98。 2.胡哲豪。2011。篩選適合台灣地區生長之海水微藻及其油脂生產之研究。大葉大學生物業科技學系碩士論文。 3.藍大鈞。2002。藻類固定二氧化碳與藻體的利

用研究。長庚大學化工與材料工程所碩士論文。 4.Abad, S. and Turon, X. 2012. Valorization of biodiesel derived glycerol as a carbon source to obtain added-value metabolites: focus on polyunsaturated fatty acids. *Biotechnol. Adv.* 30: 733-741 5.An, J. Y., Sim, S. J., Lee, J. S. and Kim, B. W. 2003. Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*, *J. Appl. Phycol.* 15: 185-191. 6.Anderson, R.A. 2005. *Algal Culturing Techniques*. Academic Press Publication, West Boothbay Harbor. 7.Apt, K.E. and Behrens, P.W. 1999. Commercial developments in microalgal biotechnology. *J. Phycol.* 35: 215-226. 8.Aslan, S. and Kapdan, I.K. 2006. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol. Eng.* 28: 64 – 70. 9.Banerjee, A., Sharma, R., Chisti, Y. and Banerjee, U. C. 2002. *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. *Crit Rev Biotechnol.* 22: 245-279. 10.Barbosa, M.J., Janssen, M., Ham, N., Tramper, J. and Wijffels, R.H. 2003. Microalgae cultivation in air-lift reactors: modeling biomass yield and growth rate as a function of mixing frequency. *Biotechnol Bioeng.* 82(2): 170 – 9. 11.Becker, E.W. 1994. *Microalgae: biotechnology and microbiology*. Cambridge University Press, New York. 12.Benemann, J.R., Koopman, B.L., Weissman, J.C. and D.M. 1978. Eisenberg and W.J. Oswald, An Integrated System for the Conversion of Solar Energy with Sewage-grown Microalgae, Report, Contract D(0-3)-34, U.S. Dept. of Energy, SAN-003-4-2, 13.Bhatnagar, A., Chinnasamy, S., Singh, M. and Das, K.C. 2011. Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. *Applied Energy.* 88: 3425-3431. 14.Borowitzka, M.A., 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J. Biotechnol.* 70, 313 – 321. 15.Boyle, N.R. and Morgan, J.A. 2009. Flux balance analysis of primary metabolism in *Chlamydomonas reinhardtii*. *BMC Syst. Biol.* 3: 4. 16.Brautigam, K., Dietzel, L., Kleine, T., Stroher, E., Wormuth, D., Dietz, K.J., Radke, D., Wirtz, M., Hell, R., Dormann, P., Nunes-Nesi, A., Schauer, N., Fernie, A.R., Oliver, S.N., Geigenberger, P., Leister, D. and Pfanschmidt, T. 2009. Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in *Arabidopsis*. *Plant Cell.* 21: 2715 – 2732. 17.Brennan, L. and Owende, P. 2010. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sust. Energy Rev.* 14: 557 – 577. 18.Brown, M. R., Dunstan, G. A., Jeffrey, S. W., Volkman, J. K., Barrett, S. M. and LeRoi, J. M. 1993. The influence of irradiance on the biochemical composition of the prymnesiophyte *Isochrysis* sp. (Clone T-ISO). *J Phycol* 29: 601-612. 19.Canovas, S., Picot, B., Casellas, C., Zulkifi, H., Dubois, A. and Bontoux, J. 1996. Seasonal development of phytoplankton and zooplankton in a high-rate algal pond. *Water Sci. Technol.* 33: 199 – 206. 20.Casadevall, E., Dif, D., Largeau, C., Gudim, C., Chaumont, D. and Desanti. 1985. Studies on batch and continuous cultures of *Botryococcus braunii*: Hydrocarbon production in relation to physiological state, cell ultrastructure and phosphate nutrition, *Biotechnol. Bioeng.* 27: 286-295. 21.Chandra, R. and Venkata Mohan, S. 2011. Microalgal community and their growth conditions influence biohydrogen production during integration of dark-fermentation and photo-fermentation processes. *Int. J. Hydrogen Energy* 36: 12211 – 12219. 22.Chang, H.N. 2009. Biofuels production from volatile fatty acid platform. *Bioenergy II.* 23.Chang, H.N., Kim, N.J. and Kang, J.W., Jeong, C.M. 2010. Biomass-derived volatile fatty acid platform for fuels and chemicals. *Biotechnol. Bioprocess Eng.* 15: 1 – 10. 24.Chen, Y.C. and Lee, M.C., 2012. Double-power double-heterostructure light-emitting diodes in microalgae, *Spirulina platensis* and *Nannochloropsis oculata*, cultures. *J. Mar. Sci. Technol.* 20(2):233-236. 25.Chen, Y., Deng, S., Hennessy, K., Lin, X., Lui, Y., Wang, Y., Martinez, B. and Ruan, R. 2009. Review of the biological and engineering aspects of algae to fuels approach. *Int. J. Agric. Biol. Eng.* 2(24):1-30. 26.Chi, Z., Liu, Y., Frear, C. and Chen, S. 2009. Study of a two-stage growth of DHA-producing marine algae *Schizochytrium limacinum* SR21 with shifting dissolved oxygen level. *Appl Microbiol Biotechnol.* 81:1141 – 8. 27.Chini, Z. G., Lavista, F., Bastianini, A., Rodolfi L., Vincenzini M. and Trevisan MR. 1999. Production of eicosapentaenoic acid by *Nannochloropsis* sp. cultures in outdoor tubular photobioreactors. *J Biotech.* 70: 299 – 312 28.Chinnasamy, S., Bhatnagar, A., Hunt, R.W. and Das, K.C. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresour. Technol.* 101: 3097 – 3105. 29.Chiu, S.Y., Kao, C.Y., Chen, C.H., Kuan, T.C., Ong, S.C. and Lin, C.S. 2008. Reduction of CO₂ by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. *Bioresour Technol.* 99: 3389 – 96. 30.Chojnacka, K. and Marquez-Rocha, F.J. 2004. Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. *Biotechnology.* 3: 21 – 34. 31.Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P. and Borghi, M.D. 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chem. Eng. Process.* 48 (6): 1146 – 1151. 32.Cook, J.R., 1967. Photoassimilation of acetate by an obligately phototrophic strain *Euglena gracilis*. *J. Protozool.* 14: 382-384. 33.Cook, J.R., 1968. The cultivation and growth of *Euglena*. In: Buetow, D.E. (Ed.), *The Biology of Euglena*. Academic Press, New York, USA. 1: 243-314. 34.Courchesne, N.M.D., Parisien, A. and Wang, B. 2009. Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. *J. Biotechnol.* 141: 31 – 41. 35.Cresswell, R.C., Rees, T.A.V. and Shah, N. 1989. *Algal and Cyanobacterial Biotechnology*. Longman Scientific and Technical, New York. 36.Cuaresma, M., Janssen, M., Vilchez, C. and Wijffels, R.H. 2011. Horizontal or vertical photobioreactors? How to improve microalgae photosynthetic efficiency. *Bioresour Technol.* 102: 5129 – 37. 37.Das, P., Aziz, S.S. and Obbard, J.P. 2011. Two phase microalgae growth in the open system for enhanced lipid productivity. *Renewable Energy.* 36 (9): 2524 – 2528 38.De la Noue, J., Lessard, P. and Proulx, D. 1993. Tertiary treatment of secondarily treated urban wastewater by intensive culture of *Phormidium bohneri*. *Environ. Technol.* 15: 449 – 458. 39.de Morais, M.G. and Costa, J.A. 2007a. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Convers. Manage.* 48: 2169 – 2173. 40.de-Bashan, L.E. and Bashan, Y., 2004. Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997 – 2003). *Water Res.* 38: 4222 – 4246. 41.Degen, J., Uebele, A., Retze, A., Schmid, S.U. and Trosch, W. 2001. A novel air lift photobioreactor with baffles for improved light utilization through the flashing light effect. *J Biotechnol.* 92: 89 – 94 42.Devi, M.P. and Venkata Mohan, S. 2012. CO₂ supplementation to domestic

wastewater enhances microalgae lipid accumulation under mixotrophic microenvironment: effect of sparging period and interval. *Bioresour. Technol.* 112: 116 – 123. 43. Doran, P.M. 1995. *Bioprocess engineering principles*. Published by Academic Press. 44. Doucha, J., Straka F. and Livansky, K. 2005. Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. *J. Appl. Phycol.* 17: 403 – 412. 45. Dragone, G., Fernandes, B., Vicente, A. and Teixeira, J.A. 2010. Third generation biofuels from microalgae. In: Vilas, A.M. (Ed.), *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. Formatex Research Center, Badajoz. 2: 1355 – 1366. 46. Dutta, D., De, D., Chaudhuri, S. and Bhattacharya, S.K. 2005. Hydrogen production by Cyanobacteria. *Microb Cell Fact.* 4 (36): 1-11. 47. Falkowski, P.G. 1980. Light shade adaptation in microalgae. In: Falkowski PG, ed. *Primary Productivity in the Sea*. New York: Plenum Press. 99 – 119. 48. Farooq, W., Lee, Y.C., Ryu, B.G., Kim, B.H., Kim, H.S., Choi, Y.E. and Yang, J. W. 2013. Two-stage cultivation of two *Chlorella* sp. strains by simultaneous treatment of brewery wastewater and maximizing lipid productivity. *Bioresour. Technol.* 132: 230 – 238. 49. Fernandez, A.V., Vargas, G. and Alarcon, N. 2008. Evaluation of marine algae as a source of biogas in a two-staged anaerobic reactor system. *Biomass Bioenerg.* 32: 338 – 344. 50. Fox, R.D. *Spirulina production and potential*. France: Edisud.; 1996. ISBN 2-84744-883-X. 51. Ganuza, E., Benitez-Santana, T. and Atalah, E. 2008. *Cryptocodinium cohnii* and *Schizochytrium* sp. as potential substitutes to fisheries derived oils in *Sparus aurata* microdiets. *Aquaculture* . 277: 109-116. 52. Ganuza, E., Anderson, A.J. and Ratledge, C. 2008. High-cell-density cultivation of *Schizochytrium* sp. in an ammonium/pH-stat fed-batch system. *Biotechnol. Lett.* 30: 1559-1564. 53. Gao, C, Zhai, Y, Ding, Y and Wu, Q. 2010. Application of sweet sorghum for biodiesel production by heterotrophic microalga *Chlorella protothecoides*. *Appl Energy.* 87: 756 – 61. 54. Garcia, O.P., Bashan, Y. and Puente, M.E., 2011. Organic carbon supplementation of sterilized municipal wastewater is essential for heterotrophic growth and removing ammonium by the microalgae *Chlorella vulgaris*. *J. Phycol.* 47: 190 – 199. 55. Garcia, O.P., Froylan Escalante, M.E., de-Bashan, L.E. and Bashan, Y. 2011. Heterotrophic cultures of microalgae: metabolism and potential products. *Water Res.* 45: 11 – 36. 56. Geider, R.J. and La Roche, J. 2002. Redfield revisited: variability of C : N : P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37: 1 – 17. 57. Gray, N.F. 1989. *Biology of Wastewater Treatment*. Oxford Univ. Press, Oxford. 58. Han, X., Miao, X.L. and Wu, Q.Y. 2006. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *J. Biotechnol.* 126: 499 – 507. 59. Hanagata, N. 1992. Tolerance of microalgae to high CO₂ and high temperature. *Phytochemistry* 31: 3345 – 3348. 60. Harwood, J.L. 1998. Membrane lipids in algae. In: Siegenthaler, P.A., Murata, N. (Eds.), *Lipids in Photosynthesis: Structure, Function and Genetics*. Kluwer Academic Publishers., Kluwer, Netherlands. 61. Hoshida, H., Ohira, T., Minematsu, A., Akada, R. and Nishizawa, Y. 2005. Accumulation of eicosapentaenoic acid in *Nannochloropsis* sp. in response to elevated CO₂ concentrations. *Journal of Applied Phycology* 17: 29 – 34. 62. Hsieh, C.H. and Wu, W.T. 2009. Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. *Bioresour. Technol.* 100: 3921 – 3926. 63. Huang, G.H., Chen, F., Wei, D., Zhang, X.W. and Chen, G., 2010. Biodiesel production by microalgal biotechnology. *Appl. Energy* 87: 38-46. 64. Ibraheem, I.B.M. 1998. Utilization of certain algae in the treatment of wastewater. Ph.D. Thesis, Fac. of Sci. Al-Azhar Univ., Cairo, Egypt, pp. 197. 65. Illman, A.M., Scragg, A.H. and Shales, S.W. 2000. Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. *Enzyme and Microbial Technology.* 27: 631 – 635. 66. Ip, S.Y., Bridger, J.S., Chin, C.T., Martin, W.R.B. and Raper, W.G.C. 1982. Algal growth in primary settled sewage – the effects of five key variables. *Water Res.* 16: 621 – 632. 67. Janssen, M., Tramper, J., Mur, L.R. and Wijffels, R.H. 2003. Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up, and future prospects. *Biotechnol Bioeng.* 81(2): 193 – 210. 68. Kaewpintong, K., Shotipruk, A., Powtongsook, S. and Pavasant, P. 2007. Photoautotrophic high-density cultivation of vegetative cells of *Haematococcus pluvialis* in airlift bioreactor. *Bioresour. Technol.* 98: 288 – 295. 69. Kaplan, D., Christiaen, D. and Arad, S. 1988. Binding of heavy metals by algal polysaccharides. In: Stadler, T., Mollion, J., Verdus, M.C., Karamanos, Y., Morvan, H., Christiaen, D. (Eds.), *Algal Biotechnology*, Elsevier Applied Science, London, pp. 179 – 187. 70. Khotimchenko, S.V. and Yakovleva, I.M. 2005. Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance. *Phytochemistry.* 66: 73 – 79. 71. Khozin-Goldberg, I. and Cohen, Z. 2006. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneus*. *Phytochemistry.* 67: 696 – 701. 72. Kitaya, Y., Xiao, L., Masuda, A., Ozawa, T., Tsuda, M. and Omasa, K., 2008. Effects of temperature, photosynthetic photon flux density, photoperiod and O₂ and CO₂ concentrations on growth rates of the symbiotic dinoflagellate, *Amphidinium* sp.. *J. Appl. Phycol.* 20: 737 – 742. 73. Koc, Y., Peker, D., and Osmanoglu, A. 2009. Supporting teacher professional development through online video case study discussions: An assemblage of preservice and inservice teachers and the case teacher. *Teaching and Teacher Education: An International Journal of Research and Studies.* 25(8): 1158-1168. 74. Kojima, E., Zhang, K. 1999. Growth and hydrocarbon production by microalga *Botryococcus braunii* in bubble column photobioreactor. *J. Biosci. Bioeng.* 87: 811 – 817. 75. Kong, W.B., Song, H., Hua, S.F., Yang, H., Yang, Qi. and Xia, C.G. 2012. Enhancement of biomass and hydrocarbon productivities of *Botryococcus braunii* by mixotrophic cultivation and its application in brewery wastewater treatment. *Afr. J. Microbiol. Res.* 6: 1489 – 1496. 76. Kumar, D., Rai, J. and Gaur, J.P. 2012. Removal of metal ions by *Phormidium bigranulatum* (Cyanobacteria)-dominated mat in batch and continuous flow systems. *Bioresour. Technol.* 104: 202 – 207. 77. Lardon, L., Helias, A., Sialve, B., Steyer, J.P. and Bernard, O. 2009. Life cycle assessment biodiesel production from microalgae. *Environ. Sci. Technol.* 43: 6475 – 6481. 78. Lau, P.S., Tam, N.F.Y. and Wong, Y.S. 1995. Effect of algal density on nutrient removal from primary settled wastewater. *Environ. Pollut.* 89: 59 – 66. 79. Lee, C. and Palsson, B. 1996. Photoacclimation of *Chlorella vulgaris* to red light from light-emitting diodes leads to autospore release following each cellular division. *Biotechnol. Prog.* 12(2): 249-256. 80. Lee, R.E. 1980. *Phycology*. New York: Cambridge University Press. 81. Lee, Y.K, Tan, H.M, Low, C.S. 1989. Effect of salinity of medium on cellular fatty acid composition of marine alga *Porphyridium cruentum* (Rhodophyceae). *J Appl Phycol.* 1:19 – 23. 82. Li, X., Hu, H.Y. and Yang, J. 2010d.

Enhancement Effect of Ethyl-2-methyl acetoacetate on Triacylglycerols Production by a Freshwater Microalga, *Scenedesmus* sp. LX1. *Bioresour. Technol* 101: 9819 – 9821.

83.Li, X., Hu, H.Y. and Yang, J. 2010c. Lipid Accumulation and nutrients removal properties in secondary effluent of a newly-isolated freshwater microalga *Scenedesmus* sp. LX1. *New Biotechnol* 27 (1): 59 – 63.

84.Li, X., Hu, H.Y., Gan, K. and Sun, Y. X. 2010. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. *Bioresource Technology*. 101(14): 5494 – 5500.

85.Li, X., Hu, H.Y., Gan, K. and Yang, J. 2010b. Growth and nutrients removal properties 100 of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. *Ecol. Eng.* 36: 379 – 381.

86.Li, X., Hu, H.Y. and Yang, J. 2010. Lipid accumulation and nutrients removal properties in secondary effluent of a newly-isolated freshwater microalga *Scenedesmus* sp. LX1. *New Biotechnol*. 27: 59 – 63.

87.Li, Y., Horsman, M., Wang, B., Wu, N. and Lan, Q.C. 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Appl. Microbiol. Biotechnol.* 81: 629 – 636.

88.Li, Y.T., Han, D.X., Sommerfeld, M. and Hu, Q. 2011. Photosynthetic carbon partitioning and lipid production in the oleaginous microalga *Pseudochlorococcum* sp. (*Chlorophyceae*) under nitrogen-limited conditions.. *Bioresour. Technol.* 102: 123 – 129.

89.Liang, Y., Sarkany, N. and Cui, Y. 2009. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol. Lett.* 31 (7): 1043 – 1049.

90.Liang, Y., Sarkany, N. and Cui, Y. 2009. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol. Lett.* 31 (7): 1043 – 1049

91.Liang, Y.N., Cui, Y., Trushenski, J. and Blackburn, J.W. 2010. Converting crude glycerol derived from yellow grease to lipids through yeast fermentation. *Bioresour. Technol.* 101: 7581 – 7586.

92.Liang, Y.N., Sarkany, N. and Cui, Y. 2009. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol. Lett.* 31: 1043-1049

93.Lim, S., Chu, W. and Phang, S. 2010. Use of *Chlorella vulgaris* for bioremediation of textile wastewater. *J. Bioresour. Technol.* 101: 7314 – 7322.

94.Lin, Y.H., Chang, F.L., Tsao, C.Y., Leu, J.Y. 2007. Influence of growth phase and nutrient source on fatty acid composition of *Isochrysis galbana* CCMP 1324 in a batch photoreactor. *Biochemical Engineering Journal* 37: 166 – 176.

95.Lincoln, E.P. and Hill, D.T. 1980. An integrated microalgae system. In: Shelef, G., Soeder, C.J. (Eds.), *Algae biomass*, pp. 229 – 243.

96.Liu, Z.Y., Wang, G.C. and Zhou, B.C. 2008. Effect of iron of growth and lipid accumulation in *Chlorella vulgaris*. *Bioresour. Technol.* 99: 4717 – 4722.

97.Loubiere, K., Olivo, E., Bougaran, G., Pruvost, J., Robert, R. and Legrand, J. 2009. A new photobioreactor for continuous microalgal production in hatcheries based on external-loop airlift and swirling flow. *Biotechnol Bioeng.* 102(1): 132 – 47.

98.Marquez, F.J., Sasaki, K., Kakizono, T., Nishio, N. and Nagai, S. 1993. Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. *Journal of Fermentation and Bioengineering.* 76: 408-410.

99.Masseret, E., Amblard, C., Bourdier, G. and Sargos, D. 2000. Effects of a waste stabilization lagoon discharge on bacterial and phytoplanktonic communities of a stream. *Water Environ. Res.* 72: 285 – 294.

100.Mata, T.M., Martins, A.A. and Caetano, N.S. 2010. Microalgae for biodiesel production and other applications: a review. *Renew. Sust. Energ. Rev.* 14: 217-232.

101.Matsudo, M.C., Bezerra, R.P., Sato, S., Perego, P., Converti, A. and Carvalho, J.C.M. 2009. Repeated fed-batch cultivation of *Arthrospira* (*Spirulina*) *platensis* using urea as nitrogen source. *Biochem. Eng. J.* 43: 52 – 57.

102.Maxwell, D.P., Falk, S., Trick, C.G. and Huner, N.P.A. 1994. Growth at low temperature mimics high-light acclimation in *Chlorella vulgaris*. *Plant Physiol.* 105: 535 – 543.

103.Mazzuca Sobczuk, T., Garc? a Camacho, F., Molina Grima, E. and Chisti, Y. 2006. Effects of agitation on the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum*. *Bioproc. Biosyt. Eng.* 28: 243 – 250.

104.Meng, T., Gao, X., Zhang, J., Yuan, J. Y., Zhang, Y. Z. and He, J. S. 2009. “ Graft copolymers prepared by atom transfer radical polymerization (ATRP) from cellulose, ” *Polymer.* 50(2): 447-454.

105.Mengel, K., Robin, P. and Salsac, L. 1983. Nitrate reductase activity in shoots and roots of maize seedlings as affected by the form of nitrogen nutrition and the pH of the nutrient solution. *Plant Physiol.* 71: 618 – 622.

106.Mengel, K., Robin, P. and Salsac, L. 1983. Nitrate reductase activity in shoots and roots of maize seedlings as affected by the form of nitrogen nutrition and the pH of the nutrient solution. *Plant Physiol.* 71: 618 – 622.

107.Miao, X.L. and Wu, Q.Y. 2006. Biodiesel production from heterotrophic microalgal oil. *Bioresour. Technol.* 197: 841 – 846.

108.Miron, A.S., Gomez, A.C., Camacho, F.G., Grima, E.M. and Chisti, Y. 1999. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. *J Biotechnol.* 70:249 – 70.

109.Moheimani, N.R. 2005. The culture of Coccolithophorid Algae for carbon dioxide bioremediation. PhD thesis. Murdoch University.

110.Molina Grima, E. 1999. Microalgae, mass culture methods. In: Flickinger MC, Drew SW, editors. *Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation.* 3: 1753 – 69.

111.Morris, I. 1974. Nitrogen assimilation and protein synthesis. In: Stewart, W.D.P. (Ed.), *Algal Physiology and Biochemistry.* Blackwell Scientific Publications, Oxford, UK, pp. 513-613.

112.Mulbry, W., Kondrad, S. and Buyer, J. 2008. Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. *J. Appl. Phycol.* 20: 1079 – 1085.

113.Mulvenna, P.F. and Savidge, G. 1992. A modified manual method for the determination of urea in seawater using diacetylmonoxime reagent. *Estuarine, Coastal and Shelf science.* 34: 429-438.

114.Munoz, R. and Guieysse, B. 2006. Algal-bacterial processes for the treatment of hazardous contaminants: a review. *Water Resource.* 40 (15): 2799-2815.

115.Muradyan, E.A., Klyachko-Gurvich, G.L., Tsoglin, L.N., Sergeyenko, T.V. and Pronina, N.A. 2004. Changes in lipid metabolism during adaptation of the *Dunaliella salina* photosynthetic apparatus to high CO₂ concentration. *Russian Journal of Plant Physiology* 51:53 – 62.

116.Muradyan, E.A., Klyachko-Gurvich, G.L., Tsoglin, L.N., Sergeyenko, T.V. and Pronina, N.A. 2004. Changes in lipid metabolism during adaptation of the *Dunaliella salina* photosynthetic apparatus to high CO₂ concentration. *Russian Journal of Plant Physiology* 51: 53 – 62.

117.Murata, N., Takahashi, S., Nishiyama, Y. and Allakhverdiev, S.I. 2007. Photoinhibition of photosystem II under environmental stress. *Biochim. Biophys. Acta.* 1767: 414 – 421.

118.Nagase, H., Yoshihara, K., Eguchi, K., Okamoto, Y., Murasaki, S., Yamashita, R., Hirata, K. and Miyamoto, K. 2001. Uptake pathway and continuous removal of nitric oxide from flue gas using microalgae. *Biochem Engin J.* 7:241-246.

119.Nixon, P.J., Michoux, F., Yu, J., Boehm, M. and Komenda, J. 2010. Recent advances in understanding the assembly and repair of photosystem II. *Ann. Bot.* 106: 1 – 16. 120.Oh-Hama, T., and Miyachi, S. 1992. *Chlorella*. In *Micro-algal biotechnology*. Edited by M.A. Borowitzka and L.J. Borowitzka. Cambridge University Press, Cambridge, U.K. pp. 3-26. 121.Oswald, W.J. 1988. Micro-algae and wastewater treatment. In: Borowitzka, M.A., Borowitzka, L.J. (Eds.), *Micro-algal Biotechnology*. Cambridge University Press, Cambridge, UK, pp. 305 – 328. 122.Park, J.B.K, Craggs, R.J. and Shilton, A.N. 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresour Technol.* 102(1): 35 – 42. 123.Parmar, A., Singh, N.K., Pandey, A., Gnansounou, E. and Madamwar, D. 2011. Cyanobacteria and microalgae: a positive prospect for biofuels. *Bioresour. Technol.* 102: 10163 – 10172. 124.Phang, S.M. 1991. The use of microalgae to treat agro-industrial wastewater. In: *Proceedings of a Seminar held at Murdoch Univ., Western Australia, 29th, November*. 125.Phang, S.M. and Ong, K.C. 1988. Algal biomass production on digested palm oil mill effluent. *Biological Wastes.* 25: 177 – 191. 126.Posten, C. 2009. Design principles of photo-bioreactors for cultivation of microalgae. *Eng Life Sci.* 9:165 – 77. 127.Pouliot, Y., Talbot, P. and De la Noue, J. 1986. Biotraitement du purin de pore par production de biomass. *Entropie.* 130 (131) : 73 – 77. 128.Prathima Devi, M. and Venkata, M. 2012. CO₂ supplementation to domestic wastewater enhances microalgae lipid accumulation under mixotrophic microenvironment: effect of sparging period and interval. *Bioresour. Technol.* 112: 116 – 123. 129.Pruvost, J., Van Vooren, G., Cogne, G. and Legrand, J. 2009. Investigation of biomass and lipids production with *Neochloris oleoabundans* in photobioreactor. *Bioresour. Technol.* 100: 5988 – 5995. 130.Qin, J. 2005. Bio-hydrocarbons from algae: impacts of temperature, light and salinity on algae growth. Rural industries research and development corporation report. Adelaide, Australia. 131.Rao, A.R., Dayananda, C., Sarada, R., Shamala, T.R. and Ravishankar, G.A. 2007. Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Bioresour. Technol.* 98: 560 – 564. 132.Ratledge, C. 2004. Fatty acid biosynthesis in microorganisms being used for single cell oil production. *Biochimie* 86: 807 – 815. 133.Ratledge, C. and Cohen, Z. 2008. Microbial and algal oils: do they have a future for biodiesel or as commodity oils? *Lipid Technol.* 20: 155-60. 134.Ratledge, D.K., Kidd, E.A. and Beighton, D. 2001. A clinical and microbiological study of approximal carious lesions. Part 2: efficacy of caries removal following tunnel and class II cavity preparations. *Caries Res.* 35: 8-11. 135.Reitan, K. I., Rainuzzo, J. R. and Olsen, Y. 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. *J Phycol* 30: 972-979. 136.Renaud, S.M. and Parry, D.L. 1994. Microalgae for use in tropical aquaculture II: effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. *J Appl Phycol.* 6:347 – 356. 137.Renaud, S.M., Parry, D.L. and Thinh, L.V. 1994. Microalgae for use in tropical aquaculture. 1. Gross chemical and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. *J. Appl. Phycol.* 6 (3) : 337 – 345. 138.Richardson, K., Beardall, J. and Raven, J.A. 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytol.* 93: 157 – 191. 139.Richmond, A. 2004. *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd. 140.Rivkin, R.B. 1989. Influence of irradiation and spectral quality on carbon metabolism of phytoplankton. I. Photosynthesis, chemical composition and growth. *Mar. Ecol. Prog. Ser.*, 55: 291-304. 141.Robert, B. W., 1960. THE TOTAL SYNTHESIS OF CHLOROPHYLL. *Tetrahedron*, 46 (22): 7599-7659. 142.Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N. and Bonini, G. 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 – 12. 143.Rodrigues, A.M. and Oliveira, J.F.S. 1987. Treatment of wastewaters from the tomato concentrate industry in high rate algal ponds. *Water Sci. Technol.* 19: 43 – 49. 144.Ruiz-Marin, A., Mendoza-Espinosa, L.G. and Stephenson, T. 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresour. Technol.* 101:58 – 64. 145.Sambrook, J., Fritsch, E.F. and Maniatis, T. N.Y. 1989. *Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press*, 1659 p. ISBN 0-87969-309-6. 146.Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J. and Smith, A.G. 2010. Biodiesel from algae: challenges and prospects. *Curr. Opin. Biotechnol.* 21: 277 – 286. 147.Seyfabadi, J., Ramezani, Z. and Khoeyi, Z.A. 2010. Protein, fatty acid, and pigment content of *Chlorella vulgaris* under different light regimes. *Journal of Applied Phycology.* 23: 721-749. 148.Seyfabadi, J., Ramezani, Z. and Khoeyi, Z.A. 2011. Protein, fatty acid, and pigment content of *Chlorella vulgaris* under different light regimes. *Journal of Applied Phycology.* 23(4): 721-726. 149.Sforza, E., Cipriani, R., Morosinotto, T., Bertuccio, A. and Giacometti, G.M. 2012. Excess CO₂ supply inhibits mixotrophic growth of *Chlorella protothecoides* and *Nannochloropsis salina*. *Bioresour. Technol.* 104: 523 – 529. 150.Shi, X. M., Liu, H. J., Zhang, X. W. and Chen, F. 1999. Production of biomass and lutein by *Chlorella protothecoides* at various glucose concentrations in heterotrophic cultures. *Process Biochem.* 34: 341-347. 151.Shi, X.M., Zhang, X.W. and Chen, F. 2000. Heterotrophic production of biomass and lutein by *Chlorella protothecoides* on various nitrogen sources. *Enzyme Microb Technol.* 27: 312 – 8. 152.Shi, X.M., Zhang, X.W. and Chen, F. 2000. Heterotrophic production of biomass and lutein by *Chlorella protothecoides* on various nitrogen sources. *Enzyme Microb. Technol.* 27: 312-318. 153.Shu, C.H., Tsia, C.C., Liao, W.H., Chen, K.Y. and Huang, H.C. 2012. Effects of light on the accumulation of oil in a mixed culture of *Chlorella sp.* and *Saccharomyces cerevisiae*. *J. Technol. Biotechnol.*, 87: 601-607. 154.Silva, H. J. and Pirt, S. J. 1984 . Carbon Dioxide Inhibition of Photosynthetic Growth of *Chlorella*. *Journal of General Microbiology.* 130: 2833-2838. 155.Singh, R.N., Sharma S. 2012. Development of suitable photobioreactor for algae production – A review. *Renewable and Sustainable Energy Reviews* 16: 2347 – 2353. 156.Soletto, D., Binaghi, L., Lodi, A., Carvalho, J.C.M. and Converti, A., 2005. Batch and fedbatch cultivation of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. *Aquaculture.* 243: 217 – 224. 157.Solovchenko, A.E., Khozin-Goldberg, I., Didi-Cohen, S., Cohen, Z. and Merzlyak, M.N. 2008. Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga *Parietochloris incisa*. *J Appl Phycol* 20:245 – 251 158.Spoladore, P., Joannis-Cassan, C., Duran, E., and Isambert, A. 2006. Commercial application of microalgae. *Journal of Bioscience and Bioengineering* 101: 87 – 96. 159.Stein, E.D. 1973. *Isolation and Culture of Algae*, Handbook

of Phycological Methods. Culture methods and growth measurements. Cambridge University Press. 160. Suh, I.S. and Lee, C.G. 2003. Photobioreactor engineering: design and performance. *Biotechnol. Bioprocess Eng.* 8: 313-321. 161. Sukenik, A. and Livne, A. 1991. Variations in lipid and fatty acid content in relation to acetyl CoA carboxylase in the marine Prymnesiophyte *Isochrysis galbana*. *Plant Cell Physiology* 32: 371 – 378. 162. Sukenik, A. and Wahnou, R. 1991. Biochemical quality of marine unicellular algae with special emphasis on lipid composition. I. *Isochrysis galbana*. *Aquaculture* 91: 61 – 72. 163. Sukenik, A., Carmeli, Y. 1990. Lipid synthesis and fatty acid composition in *Nannochloropsis* sp. (Eustigmatophyceae) grown in a light-dark cycle. *J. Phycol.* 26: 463 – 469. 164. Takagi, M. and Karseno, Y.T. 2006. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. *J. Biosci Bioeng.* 101: 223 – 226. 165. Tam, N.F.Y. and Wong, Y.S. 1989. Wastewater nutrient removal by *Chlorella pyrenoidosa* and *Scenedesmus* sp. *Environ. Pollut.* 58: 19 – 34. 166. Tang, E.P.Y., Vincent, W.F., Proulx, D., Lessard, P. and de la Nou e, J. 1997. Polar cyanobacteria versus green algae for tertiary waste-water treatment in cold climates. *J. Appl. Phycol.* 9: 371 – 381. 167. Templeton, L., and Grady, C. 1988. Effect of culture history on the determination of biodegradation kinetics by batch and fed batch techniques. *Journal of the Water Pollution Control Federation.* 60(5) : 651 – 658. 168. Thauer, R.K., Jungermann, K. and Decker, K. 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* 41: 100 – 180. 169. Tortora, G.J., Funke, B.R. and Case, C.L. 2005. *Microbiologia.* 8a ed. Artmed, ISBN 853630488X, Porto Alegre. 170. Travieso, L., Benitez, F. and Dupeiron, R. 1992. Sewage treatment using immobilized microalgae. *Bioresour. Technol.*