Chemical Concepts

International Journal of Chemical Concepts
ISSN:2395-4256 www.chemconsai.com
Vol.01, No.02, pp 94-102, 2015

Locally Isolated Microalgae as a Source of Biodiesel and By-Products: An Integral Study of Med-Algae Project

Nagwa G-E. Mohammady[1], Heba S. El-Sayed[2], Eman M. Fakhry[1], Hala M. Taha[2], Jihan H. Mohamed[1], Nirouz H. Mahmoud[1], and Basma A. Abdelsalam[1]

[1]Botany and Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
[2]National Institute of Oceanography and Fisheries, Alexandria, Egypt.

Abstract: An integral lab study of Med-algae project was done on locally isolated algal strain *Nannochloropsis* sp. to examine its potential as a feedstock for biodiesel and other bio-products. *Nannochloropsis* sp. was isolated and cultivated in 25L Flat Panel Photobioreactor under best growth promoting conditions of light, 300 µmol m^{-2} s^{-1}; salinity, 30 g/l; nitrate, 6 mg/l; pH, 9; and dilution, 10%. The maximum growth data was obtained during autumn, 2014. The results showed that the maximum dry wt was 3.3 g/L, while the growth rate was 1.5/day. Biochemical analyses expressed in % of the dry wt were as following: carbohydrate content, 22; Protein content, 32; while lipid content was 40%. The amount of ashes, 1.1 mg/l; chlorophyll a, 0.58 mg/l; while total carotenoids were 2.56 mg/l. Amino acid analysis indicated the presence of 15 fractions. A detailed analysis of fatty acid methyl esters comprising biodiesel was present in this study.
*This study has been organized on the basis of a submitted lab scale research component of Med-algae project.
Key Words: Biodiesel, By-Products, *Chlorella* sp.

Introduction

Rapid growth population and gradual depletion of fossil fuels are forcing governments and researchers to explore alternative sources of energy. Biomass, in general, is a source of different fuels and can be utilized as a renewable energy. Biodiesel is a resultant fuel of many different technological applications on biomass conversion. biomass sourced biodiesel is widely considered to be one of the most sustainable alternatives to fossil fuels and a viable means for energy security, environmental and economic sustainability [(1)]. Biodiesel has a lot of benefits over the traditional diesel since it is renewable, non-toxic, biodegradable, and environmentally friendly [(2)]. Chemically, biodiesel is composed of a mixture of methylated, or ethylated fatty esters [(3)].

However, utilization of biomass of food crops as a fuel is not good chose on long run strategy, particularly in developing countries. Microalgae, which embody lots of unparalleled advantages as nonfood resources, are viewed to be a promising feedstock of the third-generation biodiesel [(4), (5),(9),(6),(7),(8)].

Microalgae are photosynthetic microbes, either being prokaryotic or eukaryotic organisms, that can convert solar energy into valuable compounds. Consumption of microalgae as a source of biodiesel has a lot of benefits over food crops. They are non edible; characterized by having a short life cycle; have high photosynthetic efficiency compared to the terrestrial plants; potential to produce 10-20 times higher yield of oil compared to other oleaginous seeds or vegetable oils; can be cultivated in marginal land under harsh conditions; need a minimal input requirements; as they have the ability to control cell composition without decreasing productivity [(9), (10), (11), (12)]. Algal biomass contains 5-57% carbohydrates, 17-71% protein, and 4-40 % lipid [(13),

[14], [15]. A lot of biotechnological applications identified algal biomass refinery for food, feed, fertilization and energy production. Therefore, from an economic point of view, refinery of algal biomass can contribute for simultaneous food and energy security [16].

The project "Production of biodiesel from microalgae in selected Mediterranean Countries" Med-algae project (http://www.med-algae.eu) is a new technology project which can contribute to the goals of the European Union (EU) strategy on "Climate change and energy. The project funded by the Programme ENPI European Neighbourhood and Partnership Instrument (ENPI) - Mediterranean Sea Basin Joint Operational Programme. The consortium is consisted of 12 organisations: research organizations, academic institutions, energy agencies, private organizations from 6 countries: Cyprus, Greece, Italy, Malta, Lebanon and Egypt, table 1. Alexandria University, represented by the Faculty of Science, is a structural component of this international Mediterranean project.

As a part of Med-algae project's work plan; a study was to develop the native microalgae in each participated country to examine their potential as a source of biodiesel and other by-products. The plan was to optimize the cultivation conditions of bloom forming microalgae in each country, with its own weather, to compare the best results as to recognize the best region (s) for these applications as well. At Med-algae lab (a lab located at the Faculty of Science, Alexandria University, and funded by European Union - ENPI program) the efforts have been exerted to isolate different algal strains from different localities of the east harbour region, a rich region with algal strains of Alexandria city. Isolates were optimized under different lab conditions throughout 2013-2014. The predominant algal strain, *Nannochloropsis* sp. underwent a lot of biochemical analyses to address an answer for previous queries.

Table 1: MED-ALGAE Project partners

Institute	Country
Agricultural Research Institute (ARI) COORDINATOR	Cyprus
Cyprus Energy Agency (CEA)	Cyprus
Malta Intelligent Energy Management Agency (MIEMA)	Malta
Fondazzjoni Temi Zammit (FTZ)	Malta
Studio Sardo (SS)	Italy
National & Kapodistrian University of Athens (NKUA)	Greece
National Research Centre (NRC)	Egypt
The Lebanese Association for Energy Saving & for Environment (ALMEE)	Lebanon
Faculty of Science, Alexandria University (ALEX)	Egypt
American University of Beirut (AUB)	Lebanon
National Technical University Of Athens (NTUA)	Greece
Universita' Mediterranea Di Reggio Calabria (UMRC)	Italy

Materials and Methods

Preliminary experiments: Isolates were obtained from natural water sources at the eastern harbor of Alexandria city, in 2013. Samples were collected and stored in bags along with some seawater. Samples were brought to the Med-algae lab and grown with Guillard's f2 medium for blooming. Algal strains were grown successfully in the laboratory, and the existed strain was identified as *Nannochloropsis* sp. using both light and electron microscopy.

Chronically, *Nannochloropsis* sp. was cultivated batch-wise in one liter conical flasks under the influence of seasonal variations of 2013-2014. The best growth promoting conditions were examined and reported as follow: light was 300 µmol m^{-2} s^{-1}, with a photoperiod of 16/8 h light / dark cycles. The salinity was 30 g/l; nitrate, 6 mg/l; pH, 9; and dilution, 10%, at autumn 2014.

Growth and maintenance of culture: At the present study, *Nannochloropsis* sp. was transferred from one liter conical flask and cultivated in 25L Flat Panel Photobioreactor (FPP) with f2 medium [17] under the best growth promoting conditions mentioned previously, at autumn 2014. The dimensions of FPP are 5 x 50 x 100

cm, with a glass thickness of 10 mm. The starter algae inoculums were $18x10^6$. The illumination was on one side of FPP. The aeration was supplied through the injection of atmospheric air at the bottom of the FPP, with a temperature of 25±2°C.

Microscopic Examination: a- Light microscopy examination: a 30 microliter culture sample was provided for light microscopy (Optika, 4083.B5) examination.

b- Transmission Electron Microscopy (TEM): The algal cells were prepared from the centrifuged cultures according to [18]. Algal samples were then suspended in pure Spurr's resin for two days at 4°C in darkness before embedding in Spurr's resin [19]. The thin sections were stained according to [20].

Growth measurements and dry weight determination: Cell density was daily counted by haemocytometer, and the growth rate was calculated. At the maximum growth rate (1.5/day), the cells were harvested by the centrifuge (Hermle 2300) at 5000rpm for 10 mines, and then washes twice by distilled water. Cells were dried at 60°C in oven (XMTD-3000) until it reaches a constant weight.

Ash Determination: Ash content of the dry weigh was determined according to the NREL Laboratory Analytical Procedures [21]. Algal sample was burned in the muffle (SX3-Ceramic Fiber Muffle Furnace) at 550 °C until a constant weight.

Pigment analysis: 10 ml of algal culture was centrifuged at 5000 rpm for 10 min and the pellet was treated with 90% acetone [22,23]. The spectral analysis was carried out using the UV-spectrophotometer (UV-2005 Selecta), at E664, E630, E480 and E750 nm. Both chlorophyll a and carotenoids were calculated according to the equations below:

Chl a (mg/l) = 11.47 E664- 0.40 E630
Carotenoids (mg/l) = 4· (E 480 - E750)

Estimation of Carbohydrate: Total carbohydrate was determined using the colorimetric method of [24]. The cells were collected by centrifugation at 5,000 rpm for 10 minutes and weighed samples were mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of 95% sulfuric acid was added to the mixture. After allowing the test tubes to stand for 10 min, they are vortexed for few seconds and placed for 20 minutes in a water bath at room temperature for yellow -Orange color development. Light absorption was measured at 490 nm using the UV-spectrophotometer.

Protein content and amino acid composition: The lyophilized dry weight of the cells was washed with a phosphate buffered saline solution, and measured using the spectrophotometer at an absorbance of 750 nm [25]. The absorbance value was compared to the standard curve based on bovine serum albumin. Amino acids content was determined by acid hydrolysis method [26], and analyzed by amino acid analyzer (LC 3000, Hamburg, Germany).

Total crude lipid determination: A mixture of $CHCl_3$: MeOH (2:1, v/v) was added to the algal sample using the separating funnel. Lipid was estimated using rotary evaporator, and the weight of total lipid was calculated gravimetrically.

Estimation of algal oil: Algal oil was extracted using soxhlet with *n* hexane [28]. A sample of the dried algae was placed in the thimble inside the Soxhlet apparatus at 60 °C, for 8h. The organic solvent was evaporated by rotary evaporator, and the algal oil was finally collected and weighted.

Analysis of fatty acid methyl esters (FAMEs): Algal oil was esterified [29], and analyzed using GC, 4 cm- Schimadzu equipped with a flame ionization detector. The packing column material was SP-2340. Fatty acid fractions were identified according to the internal standards.

Results

The dominant strain of the bloom forming microalgae isolated from the east harbour region, Fig 1, was *Nannochloropsis* sp. The strain was cultivated in 25L FPP under the best growth promoting conditions, Fig. 2. Based on the light microscopy examination, Fig. 3, the cell morphology showed that the alga is a unicellular coccoid form with pico-planktonic algal size, and there is no flagella were observed. Further identification was

done using TEM (Fig. 4) showed that the strain is characterized by a multilayered cell wall. The cell has a large anterior nucleus and a single large parietal chloroplast. Fat granules were observed inside the plastid. According to the above observations we identified the strain as *Nannochloropsis* sp.

At the age of late exponential growth phase the dry weight of *N*. sp. was 3.3 g/l, and the growth rate was 1.5/day. While the biochemical analyses showed that the amounts of carbohydrate, protein, and lipid were 22, 32, and 40% of biomass, respectively. Chlorophyll *a* was 0.58 mg/l, carotenoids was 2.3 mg/l, while the ashes content was 1.1 mg/l, table 2.

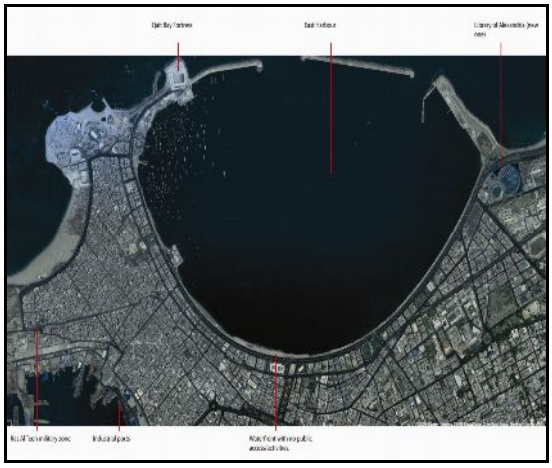

Fig. 1: Samples were collected from different localities at east harbor of Alexandria city.

Fig. 2: *Nannochloropsis* sp. cultivated in Flat Panel Photobioreactor.

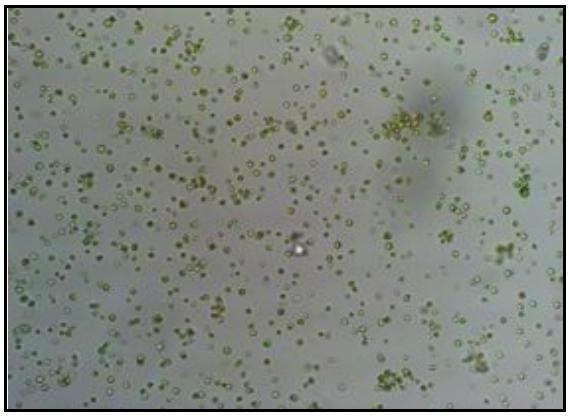

Fig 3: *Nannochloropsis* sp. under light microscopy: LM X40

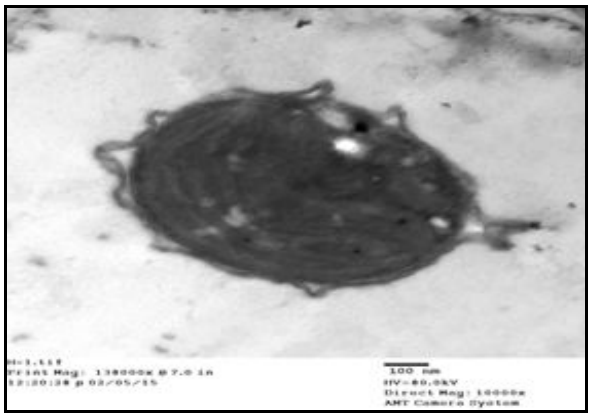

Fig 4: Ultrastructure of *Nannochloropsis* sp.

Table 2: Biomass and bio-analyses of Nannochloropsis sp.

Dry Weight (g/l)	Growth Rate	Carbohydrate% of biomass	Protein% of biomass	Lipid% of biomass	Chl. a (mg/l)	Carotenoid (mg/l)	Ashes (mg/l)
3.3	1.5 / day	22	32	40	0.58	2.3	1.1

Table 3: Amino acids composition of *Nannochloropsis.* sp., data expressed as percentage of dry wt.

Amino acids	Amount %
Glutamic	1.98
Arginine*	1.48
Proline	3.99
Histidine*	0.75
Aspartic	3.58
Threonine*	1.59
Lysine*	1.14
Isoleucine*	2.39
Methionine	0.44
Glycine	1.21
Serine	1.22
Cysteine	0.03
Alanine	3.05
Valine*	1.59
Leucine*	2.42

(*) Represents essential amino acids

The composition of amino acid (mg/100 g fresh wt.) revealed the presence of fifteen fractions in *N. sp.:* glutamine, arginine, proline, histidine, aspartic, threonine, lysine, isoleucine, methionine, glycine, serine, cysteine, alanine, valine, leucine. The proportion of each amino acid is recorded in table 3.

Analysis of FAMEs (Table 4) using GC showed that Fatty ester chain lengths are ranged from C14 to C20. The concentration of saturated fractions is less than 20%. The proportion of saturated/unsaturated fractions is around 0.2. Fatty esters are mostly with long chain unsaturated fractions. C20 acids represent more than 43%. The results demonstrated the presence of saturated fractions of C16:0 & C18:0 with correspondent concentrations of 8.2 and 3.3%, respectively. On the other hand, the monosaturated fractions of C16:1, and C18:1 were also detected with a concentration of 10.33 and 10.17%, respectively. C18:2 was detected in a concentration less than ten percent.

Table 4: Fatty acids composition of *Nannochloropsis* sp., data expressed as percent of dry wt.

FAMEs	Amount %
C14:0	3.10
C16:1	10.33
C16:0	8.20
C16:2	3.50
C16:3	3.50
C18:0	3.30
C18:1	10.17
C18:2	9.25
C18:3	5.37
C20:1	9.41
C20:2	8.68
C20:0	3.20
C20:5	22.00

Discussion

The genus *Nannochloropsis* (Eustigmatophyceae) is widely distributed in marine habitats, particularly in waters with high nutrients such as in harbor regions. In this study, *N.* sp was the dominant alga after blooming at the lab.

The study showed a relatively high yield biomass (3.3 g/l), compared to flask cultivated alga (data not shown), with a growth rate of 1.5/day. [30].

Nannochloropsis is described as a good source of high valuable compounds by many authors [31], [32], [33], [34], [35]. In addition, *Nannochloropsis* algae are promising producers of biofuel precursors and nutraceuticals and are also harvested commercially for aquaculture feed [36]. In this connection, the study here showed that the ash content is 1.1 mg/l, while the carbohydrate content of the alga is about 22% of the dry wt; 0.7 g/l. On the other hand, the amount of protein is about 32% of the dry wt;1.05 g/l. Both protein and carbohydrate are molecules that would play an important role in sustainable microalgae-based bioprocesses at large scale [37]. These highly valuable nutritional compounds are serving in human health [38], animal feeding [39], and in aquaculture industry [40], as well.

Carbohydrate accumulation is inversely proportional to the lipid production , since the lipid precursor glycerol-3-phosphate is produced by glucose catabolism [41]. However, microalgal glucose polymers produced via cellulose/starch are the predominant components in the cell walls. Both starch and most of the cell wall polysaccharides can be converted into fermentable sugars for subsequent bioethanol production via microbial fermentation [42].

According to our results, the proportion of chlorophyll a to carotenoid content is about 25%. Although this result was found in agreement with previous studies [43], [44], [45] commented on the lower amount of chl a with relatively high carotenoid content that is may be a result of high irradiance received by algal cells in FPP which lead to increase carotenoids against harmful effect of light acclimation.

Data showed a variety of amino acids produced by *N.* sp., mostly are essential fractions. Amino acid cocktails are providing an ample supply of essential amino acids to the human health [46], [47],[48] besides their important utilization in animal feeding and aquaculture industry [49], [50].

In the present study, lipid content of *N.* sp was about 40% of the dry weight; 1.3 g/l. It is well known that *Nannochloropsis* algae, in general, are good lipid producers, and therefore, they are commonly used for feeding as a nutritional source [51].

Analysis of FAMEs showed that the chain lengths of fatty esters are ranged from C14 to C20. However, the ideal length of fatty acid comprising biodiesel is ranged from C10 to C18 [52]. The concentration of saturated fractions is less than 20%, besides the long chain unsaturated fractions of C20 acids are composed of more than 43% of the total pool. Triglycerides with low amount of saturated fatty acids and high long chains PUFAs concentration could affect negatively biodiesel quality [53].

However, the presence of both palmitic acid (C16:0) and stearic acid (C18:0), which are known as the most common fatty acids contained in biodiesel [54]. Both acids give good cetane number and oxidative stability to biodiesel [55]. C16:1, C18:1, and C18:2 are also found in appropriate proportions.

Finally, biodiesel production by *N.* sp. is not practical at the economical level if the alga employed for just biodiesel production. In order to improve biodiesel fuel quality, the alga must be subjected to genetic engineering for up-regulation of fatty acid biosynthesis and/or by down-regulation of β-oxidation. However, supplementation of biodiesel with other short-chain fatty acid esters may be a good choice. Economically, the algal biomass must be processed for bio-refinery to maximize its utilization for different applications.

Acknowledgment

The current work has been carried out within the framework of “MEDALGA H.E” project financed by Cross-Border Cooperation in the Mediterranean Program (ENPI CBCMED). The authors would like to thank the project coordinator, Agricultural Research Institute (ARI/Cyprus).

References

1. Saifullah A. Z. A. , Abdul Karim M.d., Yazid A.A, Microalgae: An Alternative Source of Renewable Energy. American Journal of Engineering Research (AJER), 2014, 3(3), 330-338.Andersen R. A., Algal culturing techniques, 1st edition, PSA/ Elsevier Academic Press, USA, 2005.
2. Patil P.D. and Deng S., Optimization of biodiesel production from edible and non-edible vegetable oils, Fuel, 2009, 88, 1302–1306.
3. Knothe G., Dependence of Biodiesel Fuel Properties on the Structure of Fatty Acid Alkyl Esters, Fuel Processing Technology, 2005, 86(10), 1059-1070.
4. Cantrell K. B., Ducey T., Ro K. S., and Hunt P. G., Livestock waste-to-bioenergy generation opportunities, Bioresource Technology, 2008, 99 (17), 7941–7953.
5. Umdu E. S., Tuncer M. and Seker E., Transesterification of Nannochloropsis oculata microalga's lipid to biodiesel on Al_2O_3 supported CaO and MgO catalysts, Bioresource Technology, 2009, 100 (11), 2828–2831.
6. Scott S. A., Davey M. P., Dennis J. S. et al., Biodiesel from algae: challenges and prospects, Current Opinion in Biotechnology, 2010, 21 (3), 277–286.
7. Rawat I., Ranjith Kumar R., Mutanda T., and Bux F., Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, Applied Energy, 2011, 88(10), 3411–3424.
8. Cao H., Zhang Z., Wu X., and Miao X., Direct Biodiesel Production from Wet Microalgae Biomass of Chlorella pyrenoidosa through In Situ Transesterification, BioMed Research International, 2013, Article ID 930686, 6 pages.
9. Chisti Y., Microalgae as sustainable cell factories, Environ. Eng. Manag. J., 2006, 5, 261-274.
10. Chisti Y., Biodiesel from microalgae, Biotech. Adv., 2007, 25, 294-306.
11. Chisti Y., Biodiesel from microalgae beats bioethanol, Trends in Biotechnology, 2008, 26 (3), 126–131.
12. Verma N.M., Mehrotra S., Shukla A. and Mishra B. N., Prospective of biodiesel production utilizing microalgae as the cell factories: A comprehensive discussion, African Journal of Biotechnology, 2010, 9 (10), 1402-1411.
13. Cohen Z., Chemicals from microalgae, 1st edition. Taylor & Francis, London, 1999.
14. Richmond A., Handbook of microalgal culture, 1st edition, Blachwell Publishing, Oxford, 2004.
15. Andersen R. A., Algal culturing techniques, 1st edition, PSA/ Elsevier Academic Press, USA, 2005.
16. Verdelho V., A. P. Carvalho A. P., Fonseca D. and Navalho J., In Clemens Posten and Christian Walter (Eds.) Microalgal Biotechnology, Integration and Economy, Walter de Gruyter GmbH, Berlin/Boston, 2012.
17. Guillard R. R. L., Culture of phytoplankton for feeding marine invertebrates. In Smith, W. L. and Chanley M. H. (Eds.) Culture of Marine Invertebrate Animals, Plenum Press, New York, USA, 1975, 26-60.
18. Chen Y.C., Immobilized Isochrysis galbana (Haptophyta) for long-term storage and applications for feed and water quality control in clam (Meretrix lusoria) cultures. J. App Phycol., 2003, 15, 439-444.
19. Spurr, A. R., A low viscosity epoxy resin embedding medium for electron microscopy, J. Ultrastruct. Res., 1969, 26, 31-43.
20. Smith M. and Croft S., Embedding and thin section preparation, In Harris I. R. (Ed.), Electron Microscopy in Biology, IRL. Press, Oxford, New York, Tokyo, 1991, pp. 17-37.
21. NREL National Renewable Energy Laboratory, www.nrel.gov/biomass/ analytical_procedures.html, 2011.
22. Jeffery S.W. and Humphrey G.F., New spectrophotometric equations for determining chlorophyll a, b c1 and c2 in higher plants, algae and natural phytoplankton, Biochem Physiol. Pflanz, 1975, 167, 191-194.
23. Strickland I.D.H. and T.R. Parsons, A Practical Handbook of Seawater Analysis. 2nd Edn., Fisheries Research Board of Canada, Ottawa, 1972, pp 310.
24. DuBois M., Gilles K., Hamilton J., Rebers P., and Smith F., Colorimetric method for determination of sugars and related substances, Analytical Chemistry, 1956, 28(3), 350–356.
25. Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J., Protein measurement with the Folin phenol reagent, Journal of Biological Chemistry, 1951, 193, 265-275.
26. Speckman D. H., Stein W. H. and Moore S., Automatic recording apparatus for use in the chromatography of amino acids, Anal. Chem., 1958, 30, 1190-1205.

27. Bligh E.G. and Dyer W.J., A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 1959, 37, 911-917.
28. Topare N., Raut S., Renge v., Khedkar S., Chavan Y., and Bhagat S., Extraction of oil from algae by solvent extraction and oil expeller method, Int. J. Chem. Sci., 2011, 9 (4), 1746-1750.
29. Radwan S.S., Sources of C20 polyunsaturated of fatty acids for use, Applied Microbiology and Biotechnology, 1978, 35, 421–430.
30. Briassoulis D., Panagakis P., Chionidis M., Tzenos D. , Lalos A., Tsinos C., Berberidis K.and Jacobsen A., An experimental helical-tubular photobioreactor for continuous production of Nannochloropsis sp. Bioresource Technology, 2010, 101, 6768–6777.
31. Forjan E., Garbayo I., Casal C. and Vilchez C., Enhancement of carotenoid production in Nannochloropsis by phosphate and sulphur limitation. In: Mendez-Vilas A (ed) Communicating current research and educational topics and trends in applied microbiology, Formatex, Badajoz, 2007, pp 356–364.
32. Howe BA., Roman-Muniz IN., Willson BD., Archibeque SL. and Evaluation of algae meal from Nannochloropsis oculata as a protein source for non-ruminant animals [abstract], J Anim Sci., 2010, 88(Suppl 2), 101–102.
33. Bong S.C. and Loh S.P., A study of fatty acid composition and tocopherol content of lipid extracted from marine microalgae, Nannochloropsis oculata and Tetraselmis suecica using solvent extraction and supercritical fluid extraction, International Food Research Journal, 2013, 20(2), 721-729.
34. Hamidi N., Yanuhar U., and Wardana I. N. G., Potential and properties of marine microalgae Nannochloropsis oculata as biomass fuel feedstock, International Journal of Energy and Environmental Engineering, 2014, 5(4), 279-290.
35. Kent M., Welladsen HM., Mangott A. and Li Y., Nutritional Evaluation of Australian Microalgae as Potential Human Health Supplements, PLoS ONE, 2015, 10 (2), e0118985, doi:10.1371/journal.pone.0118985.
36. Scholz M., Taylor L. Weiss, Robert E. Jinkerson, Jing J., Roth R., Goodenough U., Matthew C. Posewitz, Henri G. Gerken (2014), Ultrastructure and Composition of the Nannochloropsis gaditana Cell Wall Eukaryotic Cell, 13(11), 1450-1464.
37. Millán-Oropeza A., Torres –Bustillos L.G. and Fernández-Linares L., Simultaneous effect of nitrate (NO3-) concentration, carbon dioxide (CO2) supply and nitrogen limitation on biomass, lipids, carbohydrates and proteins accumulation in Nannochloropsis oculata. Biofuel Research Journal, 2015, 5, 215-221.
38. Olofsson M., Lamela T., Nilsson E., Bergé J. P., Del Pino V., Uronen P., and Legrand C. Combined Effects of Nitrogen Concentration and Seasonal Changes on the Production of Lipids in Nannochloropsis oculata, Marine drugs, 2014, 12(4), 1891-1910.
39. Enzing C., Ploeg M., Barbosa M., and Sijtsma L., Microalgae-based products for the food and feed sector, an outlook for Europe, JRC Scientific and Policy Reports, European Commission, 2014.
40. Yaakob Z., Ali E., Zainal A., Mohamad M., and Takriff M. S., An overview: biomolecules from microalgae for animal feed and aquaculture.Journal of Biological Research-Thessaloniki, 2014, 21(1), 6.
41. Chen Y.C., Zhao X.Q., Yen H.W., Ho S.H., Cheng C.L., Lee D.J., Bai F.W.and Chang J.S., Microalgae-based carbohydrates for biofuel production. Biochem. Eng. J, 2013, 78, 1-10.
42. Wang X., Liu X. and Wang G., Two-stage hydrolysis of invasive algal feedstock for ethanol fermentation. J. Integr. Plant. Biol., 2011, 53, 246-252.
43. Mohammady N., Chen Y-C., El-Mahdy A., Mohammad, R. Physiological responses of the eustigmatophycean Nannochloropsis salina to aqueous diesel fuel pollution OCEANOLOGIA, 2005, 47 (1), pp. 75–92.
44. Macías-Sánchez M.D., Mantell C., Rodríguez M., Martínez de la Ossa E., Lubián L.M.and Montero O., Comparison of supercritical fluid and ultrasound-assisted extraction of carotenoids and chlorophyll a from Dunaliella salina. Talanta, 2009, 77, 948–952.
45. Kromkamp J., Beardall J., Sukenik A., Kopeck J., Masojidek J., Bergeijk S., Gabai S., Shaham E., Yamshon A., Short-term variations in photosynthetic parameters of Nannochloropsis cultures grown in two types of outdoor mass cultivation systems, Aquat. Microb. Ecol., 2009, 56, 309–322.
46. Tipton K., and Wolfe R., Exercise-induced changes in protein metabolism. Acta Physiological Scandinavica, 1998, 162, 377-87.

47. Gibala M., Dietary protein, amino acid supplements and recovery from exercise, Sports Science Exchange, 2002, 15 (4), 1-4.
48. Williams M., Dietary Supplements and Sports Performance: Amino Acids, Journal of the International Society of Sports Nutrition, 2005, 2(2), 63-67.
49. Brown M., The amino-acid and sugar composition of 16 species of microalgae used in mariculture, Journal of Experimental Marine Biology and Ecology, 1991, 145(1), 79–99.
50. Ferreira M., Coutinho P., Seixas P., Fábregas J. and Otero A., Enriching Rotifers with Premium Microalgae. Nannochloropsis gaditana . Marine Biotechnology, 2009, 11(5), 585-595.
51. Mohammady N., Total, Free and Conjugated Sterolic Forms in Three Microalgae Used in Mariculture. Z. Naturforsch, 2004, 59c, 619-624.
52. Whyte L.G., Hawari J., Zhou E., Bourbonni´ere L., Inniss W. E. and Greer C.W., Biodegradation of variable-chain length alkanes at low temperatures by a psychrotrophic Rhodococcus sp., Appl. Environ. Microbiol., 1998, 64 (7), 2578–2584.
53. Mohammady N., Characterization of the Fatty Acid Composition of Nannochloropsis salina as a Determinant of Biodiesel Properties. Z. Naturforsch, 2011, 66c, 328 – 332.
54. Lee JY., Yoo C., Jun SY., Ahn CY., Oh HM., Comparison of several methods for effective lipid extraction from microalgae, Bioresour Technol, 2010, 101, supplement, pp. 575–577.
55. Chinnasamy S., Bhatnagar A, Hunt RW. and Das KC., Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour Technol, 2010, 101, 3097–105.

*****