Seaweed Res. Utiln., 38(2) : 73 - 78, 2016

Lipid extraction and production of biodiesel from marine diatom *Chaetoceros muelleri* isolated from Vellar estuary, Southeast coast of India

SHAILESH UPADHYAY[1], KAPIL LAWRENCE[1], A.M. LALL[1], M. SURESH[2] AND P. ANANTHARAMAN[2]*
[1]*Department of Biochemistry and Biochemical Engineering, JSBB, SHIATS, Allahabad, India*
[2]*CAS in Marine Biology, Annamalai University, Parangipettai - 608 502, Tamil Nadu, India*
**E-mail : panantharaman@gmail.com*

ABSTRACT

The marine algae based biodiesel may be the greatest possible options for future energy, particularly for India where land resources and freshwater are limited. In the above context, The aim of this research is to reveal the informtion on growth and lipid content of *Chaetoceros muelleri* in different medium varying Si concentration, isolated from Vellar estuary, South east coast of India and characterized at different Si treatment as lipid production. In this experiment, *C. muelleri* showed maximum cell density in 11th day in treament T4 (Si concentration 159 µM) 19.11±0.09 × 10^{5}cells ml^{-1} while minimum cell density was found in treatment T1 (Si concentration 0 µM). Lipid content was varying at different treatments and found to be maximum 25.6 ±0.48% of dry biomass in treatment T2 (Si concentration 53 µM) and minimum 14.23± 0.33 % of dry biomass in treatment T4 (Si concentration 159 µM).Treatment T1 and T3(control), the lipid content was 20.56±0.28% and 16.74 ±0.40% of dry biomass respectively. The highest lipid content was 25.6 ±0.48% of dry biomass in treatment T2 (Si concentration 53 µM) Hence, this treatment was selected for transesterification of lipid by methanol and KOH. Subsequently, Fatty Acid Methyl Ester (FAME) analysis showed the presence of saturated fatty acids of 69.62% which is highly abundant than monounsaturated and polyunsaturated fatty acid. In the present study, the fatty acids was recorded in ranged between C16–C18, which accounts for more than 90% fatty acids being predominant components for biodiesel production making *C. muelleri* species highly suitable for further exploration and commercial biodiesel production.

Introduction

The reduction of CO_2 emissions from human activities that contribute to the greenhouse effect and as a result of global warming is widely accepted. Clean and renewable energy sources appear to be an alternative solution to replace the utilization of fossil fuel in transport and industry, as well as dealing with the problem of crude oil exhaustion (Amaro *et al.*, 2011, Sing *et al*., 2013). According to an estimate (grow diesel consortium news circular, 2008), automobiles alone contribute to 70% of the total petroleum consumptions. The country faces problems in regard to the fuel requirement for increased transportation demand. The increasing import of fuel has necessitated the search for other liquid fuels as an alternative source to diesel, which is being used in large quantities in transport, industrial and agricultural sector (Meher *et al.,* 2006). Biodiesel can be direct and immediate replacement for the liquid fuels used in transport and can also be easily integrated to the logistic systems that are operating today (Escobar *et al.,* 2009). It is an attractive substitute to fossil fuel, and it is compatible with current commercial diesel engines and has clear benefits compared to diesel fuel including enhanced biodegradation, reduced toxicity and lower emission profile (Vicente, 2009).

Diatoms are the primary constituent of the marine plankton community, typically representing more than 70 % of the total plankton and are estimated to contribute up to 40 % of the total oceanic primary production (Sumper and Brunner, 2008). Microalgae (diatoms) are considered as alternative

Received : Sep. 2016; Revised : Oct. 2016; Accepted : Nov. 2016

feedstocks for biodiesel production due to several potential advantages over oilseed crops (Behzadi and Farid, 1991; Chisti, 2007). They can be cultivated on non-arable areas without destructing forests, thereby enhancing carbon dioxide reduction efficiency. Furthermore, they can grow fast with short doubling times of normally around 1 day, enabling mass production in a small area. Lastly, they do not compete with the markets for foods and feeds. Among microalgae, diatoms were reported to have high lipid contents (Sheehan *et al.,* 1998). In addition, the lipid production by these diatoms is stimulated when they are stressed by nutrient deficiency for elements such as phosphorous, silicon, and nitrogen (Lombardi and Wangersky,1991). Many microalgae can accumulate lipids due to excessive photosynthesis and some species can accumulate certain amount of lipids under heterotrophic conditions or environmental stress, such as nutrient deficiency (Takagi *et al.,* 2000). The medium which supports the growth does not favour the increased lipid accumulation (Thajuddin *et al.,* 2015). Microalgae have attracted attention for bioenergy production because microalgae can produce oil in the cell body as well as carbohydrate and protein (Kim and Lee, 2005 and Tran *et al.,* 2010).

Marine diatoms *C. muelleri* is found to be one of the most suitable microalgal species for large-scale biomass and lipid production. Moreover, *C. muelleri* can be cultivated on large-scale in indoors and outdoors for commercial utilization (Becerra-Dorame *et al.,* 2010; Lopez-Elias *et al.,* 2005). In this study, marine diatoms *C. muelleri* were isolated and investigated the effect of different Si concentration in nutrient medium on growth and lipid production. Si treatment having maximum lipid content further used for transesterification.

Material and Methods

Isolation and Identification of marine diatom

The marine diatom *Chaetoceros muelleri* was collected from Vellar estuary, Prangipeettai, 11º29'N and Longitude 79º46'E, Tamil Nadu, southeast coast of India (Bay of Bengal). The diatom collection was made by horizontal towing of phytoplankton net (No-30 bolting silk cloth with 45 µm much size) during early morning by following the method of Mohan *et al.* (2012).The individual diatom strain was isolated using serial dilution. Then the isolated pure cultures were maintained in Guilard, f/2 (1975) medium. The diatom was examined under a zoom stereomicroscope (Olympus). The diatom was identified according to their morphological characteristics by referring standard taxonomic publications (Thomas, 1997; Venkataraman. 1939; Subrahmanyan 1946).

Cultivation of Diatom and Determination of cell density

The isolated diatom was cultivated in Guilard, f/2 (1975) medium. All the experiments were conducted in 5 litre conical flasks. The culture was provided with 12:12 dark: light cycle with 4500 lux white fluorescent lamp at 25ºC±2ºC temperature. Then the strains were examined daily for the contamination. The cell density of the isolated species was determined using a 0.1 mm deep Neubauer chamber. In this study, four Si teatments were evaluated, treatment 1(T1) consisted of the standard f/2medium without Si (0 µM Si) , T2 consisted f/2 medium with reduced Si (53 µM Si), T3 consisted f/2 medium (Si 106 µM), T4 consisted f/2 medium with increased Si (159 µM Si). T3 treated as control. All treatments were done in triplicate. All data show in mean value. In all treatments study was made on the effect of Si on cell density and lipid content. Treatment having maximum lipid yield was used further for transesterification and GC-MS analysis.

Determination of lipid content

The culture was grown at different Si treatment such as T1, T2, T3 and T4 and lipid estimation was done following the method of Floch *et al.* (1956). One gram dry algal sample was homogenized with 20 ml of chloroform/methanol (2/1). After dispersion, the whole mixture was agitated for 15-20 min in an orbital shaker at room temperature. Then the homogenate was filtered using Wattman no.1 filter paper and the solvent was washed with 0.2 volumes (4 ml for 20 ml) of water or better 0.9% NaCl solution. After vortexing, the mixture was centrifuged at low speed (2000 rpm) to separate the two phases.

Transesterification of lipid and FAME analysis

The algal lipid extracted from treatment T2 (Si concentration 53 µM) was used for transesterification into fatty acid methyl ester (FAME) by following the procedure described by Ichihara *et al.* (1996). Briefly, 10 mg of lipid was dissolved in 2 ml of hexane and 200 µl of 2 M methanolic KOH (used as catalyst). Mixture was vortexed for 2–5 min followed by a brief centrifugation. The upper hexane layer was collected for FAME analysis. Quantification of FAME was carried out using gas chromatography.

GC/MS analysis

The fatty acid composition was analysed using GC–MS. The GC–MS analysis was carried out using an Agilent 6890N gas chromatography connected to an Agilent 5973 mass selective detector at 70 EV (m/z 50–550; sources at 230°C and quadrupole at 150°C) in the electron impact mode with a HP-5 capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The oven temperature was programmed for 2 min at 160 °C and raised to 300 °C at 5 °C min^{-1} and maintained for 20 min at 300 °C. The carrier gas helium was used at a flow rate of 1.0 ml min^{-1}. The inlet temperature was maintained at 300 °C with a split ratio of 50:1. The injection volume was 1 µl, with a split ratio of 50:1.

Result and Discussion

Isolation and identification of diatoms

Marine diatom was isolated from the Vellar estuary,

© Seaweed Research and Utilisation Association

southeast coast of India and identified as *C. Muelleri* by morphologically. The isolated diatoms were cultivated in different Si concentration (T1,T2, T3 and T4 Treatment) and f/2 medium (Guilard, 1975).

Cell density and Growth curve of *C. muelleri*

Figure-1 shows four valuable data that are related to growth of *C.muelleri* in different treatment. From this Figure, it can be observed that day 4 shows the highest growth compared to other days. *C.muelleri* was experienced the maximum cell density at 11th day in treatment (T3) and treatment (T4). Table shows that initial three days growth was slow in all culture medium. The objective of growth curve of *C.muelleri* in different Si medium was observe its growth trend with different Si concentration medium and optimized conditions. Under laboratory cultured conditions, *C.muelleri* showed a short lag phase that has lasting about 24 hour in all medium. Subsequently, cells grew actively from day 3 untill day 9, whereas increases growth highest in 4th day compared to other day. During this time cell is doubling and the number of new microalgae appering per day is proportional to the present population. On day 10 untill day 13 at this phase the growth of cells entered stationary phase. At this phase growth slows as a result of nutrient depletion and accumulation of toxic products. In this phase, microalgae begin to exhaust the resources that are availble to them. In day 13 it can be observed that cells undergo dead phase whereas microalgae run out of nutrients and die off.

The most important parameter regulating algal growth are nutrient quality and quantity (Jalal *et al .,* 2012).This growth curve trend was simillar to reported by Jalal *et al.* (2012) in mass culture when stress factor (Nitrogen limitation) was introduced, there was declining of cell number of *Isochyris sp.* In this experiment maximum cell density were found in 11th day in treament T4 (Si concentration 159 µM) 19.11±0.09 × 10^{5}cells ml^{-1} while minimum cell density was found in treatment T1(Si concentration 0 µM). Figure-2 shows the maximum cell density of *C. muelleri* at different Si concentartion, based on growth highest growth in T4 (Si concentration 159 µM) while lowest in treatment T1(Si concentration 0 µM). Hemalatha *et al.* (2014) simillarly reported in *Chaetoceros simplex* that maximum cell density, 19.56 × 10^{5} cells ml^{-1} reached in 212 µM silicate concentration followed by 18.95 × 10^{5} cells ml-1 in 159 µM in 10 days aged culture. Lower concentration (50% of f/2 concentration) showed minimum cell count and actual f/2 media concentration (106 µM) showed medium cell density than other high concentrations of silicate.

Determination of lipid content and Transesterfication

The total lipid content at various Si treatments is shown in Figure-3. Lipid content was varying at different treatments. It was found to be maximum 25.6 ±0.48% of dry

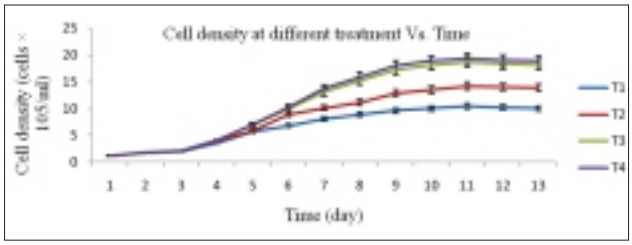

Fig. 1. *Growth curve of* C. mulleri *at different Si treatment*

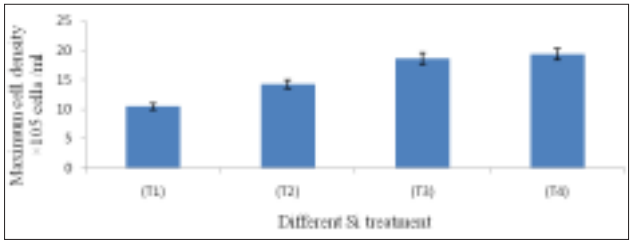

Fig. 2. *Maximum cell density of* C. mulleri *at different Si treatment*

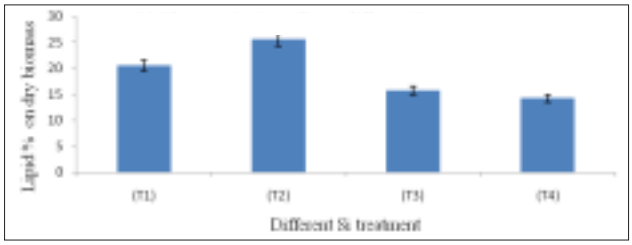

Fig. 3. *Lipid content of* C. mulleri *at different Si treatment*

biomass in treatment T2 (Si concentration 53 µM) and minimum 14.23± 0.33 % of dry biomass in treatment T4 (Si concentration 159 µM). Treatment T1 and T3(control) the lipid content was 20.56±0.28% and 16.74 ±0.40% of dry biomass respectively. Ju *et al.* (2011), reported in *Chaetoceros gracilis* that lipid content was maximum in Si depletion cell than Si deficient cell and minimum in Si rich cell. With respect to lipid content, Shalaby (2011) evaluated 17 microalgae and found the lipid content ranging from 4 to 40%; while Andrade-Nascimento *et al*. (2012) documented the lipids percentage in 12 microalgae species which ranged from 13.52 to 49.0% and 10 of them had contents from 13.52 to 28.43% very similar to the values found in the present investigation. Low levels of either sodium chloride or silicon resulted in at least 50 % increases in lipid content (Adams and Bugbee, 2013). Lipid content found maximum in treatment (T2) further this lipid used for transesterification. Synthesis of biodiesel from phytoplankton lipid was done by transesterification using hexane and methanolic KOH. After completion of transesterification reaction the upper phase was separated using separating funnel. FAME analysis was done by GC-MS.

GC-MS (FAME) analysis

The compositions of fatty acid methyl ester (FAMEs) found in *C. muelleri* are summarized in Table-1. The percentage

Table 1. *Fatty acid methyl ester content in* C.muelleri

Systemic name of FAME	Formula	Fatty acid content (%total FAME)	Family
Triacontanoic acid,	C31H62O2	1.97	SFA
Tetradecanoic acid, methyl ester	C15 H30 O2	22.44	SFA
Octadecanoic acid, methyl ester	C19 H38 O2	19.24	SFA
Pentadecanoic acid, methyl ester	C16H32O2	1.94	SFA
Hexadecanoic acid, methyl ester	C17 H34 O2	24.03	SFA
Total SFA - 69.62			
9-Hexadecenoic acid, methyl ester,	C17 H32 O2	7.97	MUFA
Hexadecanoic acid, 15-methyl-	C18 H36 O2	5.17	MUFA
11-Eicosenoic acid, methyl ester	C21 H40 O2	2.39	MUFA
Total MUFA -16.53			
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	6.83	PUFA
6,9,12-Octadecatrienoic acid, methyl ester	C19 H32 O2	1.96	PUFA
7,10,13-Eicosatrienoic acid, methyl ester	C21 H36 O2	4.29	PUFA
Methyl arachidonate	C21 H34 O2	1.76	PUFA
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19 H32 O2	1.81	PUFA
Total PUFA - 13.85			

of SFAs (69.62%) in the lipid higher than MUFAs (16.53%). Both classes were higher than polyunsaturated fatty acids (PUFAs; 13.85 %). Myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) were the most abundant SFAs. Furthermore, the triacontanoic acid and pentadecanoic acid (C15:0).Palmitoleic acid (C16:1n7), Eicosenoic aacid (C20:1) were the predominant MUFAs. PUFAs were essentially composed of linoleic acid (C18:2 n6cis), C18:3, C20:3 and C20:4. This species have FAMEs in the range of C14 to C18. C14:0, C16:0 and C18:0, C16:1, C18:1 are major component they have contributed to 81–83% of the total FAMEs content.

The growth and lipid content are the two significant components for the biodiesel production that have been quantified for a wide variety of microalgae. In the present study, the accumulation of lipid was found to be varying in *C. muelleri* at different Si content medium. It is corresponds to the previous report of Guschina and Harwood (2006) that the lipid and fatty acid composition in microalgae is species or strain specific and varies with different culture conditions. However, an additional algal characteristic for biodiesel production is the lipid suitability in terms of the type and amount produced by an algal species such as saturation, chain length and proportion of triglycerides in lipids (Griffiths and Harrison, 2009). The fatty acid profiles of *C. muelleri* are shown to contain abundance of saturated fatty acid than monounsaturated and polyunsaturated fatty acid. The content of monounsaturated fatty acid is more dominant than polyunsaturated fatty acid. This result is similar to the previous report of El-Sayed and Khairy (2013) that the fatty acid composition in *Chaetoceros Gracilis* using two different media, saturated fatty acid varies from 85.6 to 55.1%, monounsaturated fatty acid 2-32% while PUFA 12.4 to 13.9.

Chen (2012) reported 67.65 ± 3.57 % SFA, 17.68 ± 0.93 % MUFA and 14.68 % PUFA in *Cylindrotheca sp.* Small variation in this study may be due change in Si concentration (53µM) in nutrient medium. A large variation in fatty acids was observed between conditions and treatments. Increassed the saturated fatty acids and decressed the unsaturated fatty acids with decressed Si concentrations (Jiang *et al.,* 2014) .The ratio of unsaturated to saturated fatty acids has been shown to be species-specific and affected by abiotic factors (Huerlimann *et al.,* 2010; Renaud *et al.,* 2002).

The fatty acid profiles of *C. muelleri* are shown to contain fatty acids in the range between C16–C18, accounts for more than 90% fatty acids which are known to be the predominant components for biodiesel production. Another prominent characteristic of the fatty acid composition in *C.muelleri* is that the major fatty acids were found ranging from C14–C18 and these account for more than 92% of the total fatty acids and low percentage of polyunsaturated fatty acids are very significant for the potential of *C. muelleri* for the production of biodiesel (Wang *et al.,* 2014; Miao *et al.,* 2009). The content of total polyunsaturated fatty acids in *C. muelleri* is very low (10.26–16.76%). This low percentage of polyunsaturated fatty acids and predominance of shorter-chain fatty acids (defined as alkyl chains with 12–18 carbon atoms) are very significant for the potential of *C. muelleri* for the production of biodiesel (Miao *et al.,* 2009). Higher saturated fatty acids, which is ideal for biodiesel production as high proportion of poly-unsaturated fatty acids is not considered ideal for biodiesel due to potential oxidation tendency (Irshad Ahmed *et al.,* 2013).

Based on the above results, it is concluded that *C. muelleri* cell density is maximum in 159 µM Si content medium while lipid content is maximum at 53 µM Si content medium. *C.*

© Seaweed Research and Utilisation Association

muelleri is possible algal species to be used in biofuel production because of relatively high lipid 25.6 ±0.48% of dry biomass in treatment T2 (Si concentration 53 µM)and saturated fatty acid contents, specially palmitic acid, miristaic acid and stearic acid. According to parameter of saturation this species is powerful member for good quality of biodiesel production. This baseline information would be useful for the future research attention to look for more suitable conditions in order to improve lipid yield.

Acknowledgement

The authors are thankful to the authorities of SHIATS, Allahabad and CAS in Marine Biology, Annamalai University, Parangipettai for providing necessary facilities to carry out this work. They are also thankful to ICAR, New Delhi for financial support.

References

Andrade-Nascimento, I., S.S. Izabel-Marques, T.T. Domínguez-Cbanelas, S. Andrade-Pereira, J.I. Druzain, C. Oliveira de Souza, D. Vital Vich, G. Correia de Carvalho and M. Andrade Nacimento, 2012. Screening microalgae strains for biodiesel production: Lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. *Bioenerg. Res.,* 6(1): 1-13.AOAC, 2000. Official Methods of Analysis. 17th Edn.

Adams, C. and B. Bugbee 2013. Enhancing lipid production of the marine diatom *Chaetoceros gracilis*: synergistic interactions of sodium chloride and silicon. *J. Appl. Phycol.* DOI 10.1007/s10811-013-0156-7

Amaro, H. M., A.C. Guedes and F.X. Malcata 2011. Advances and perspectives in using microalgae to produce biodiesel, *Appl. Energy,* 88 (2011) : 3402–3410.

Behzadi, S. and M.M. Farid 1991. *Asia-Pac. J. Chem. Eng*., 2, 480 (2007).

Becerra-Dorame, M., J.A. Lopez-Elias and L.R. Martinez-Cordova 2010. An alternative outdoor production system for the microalgae *Chaetoceros muelleri* and Dunaliella sp. during winter and spring in Northwest Mexico. *Aquacult. Eng.,*43 (1): 24–28.

Chisti, Y. 2007. Biodiesel from microalgae, *Biotechnol. Adv*., 25(2007): 294–306.

El-Sayed, H.S. and H.M. Khairy 2013. Comparative study for biomass production and biochemical variability of two marine microalgae grown on two water sources for evaluating *Artemia franciscana. Journal of the Arabian aquaculture society.* 8(2): 385-401.

Escobar, J.C., E.S. Lora, E.S., Venturini, O.J., Yanez, E.E., Castillo, E.F., Almazan, O., 2009. Biofuels: environment, technology and food security. *Renew. Sustain. Energy Rev*., 13, 1275–1287.

Floch, J., M. Lees and G. H. Sloane-Stanley 1956. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem*., 226: 497-509.

Griffiths, M. J. and S.T.L. Harrison 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Appl. Phycol*., 21:493–507.

Guillard, R.R.L. 1975. Culture of Phytoplankton for feeding marine invertebrates. In:Smith, W.L., Chanley, M.H.(Eds), Culture of marine invertebrates animals. Plenum Press, New York. 338pp.

Guschina, I.A. and J.I. Harwood 2006. Lipids and lipid metabolism in eukaryotic algae. *Progress Lipid Research,* 45:160-186.

Huerlimann, R., R. de Nys and K. Heimann 2010. Growth, lipid content, productivity,and fatty acid composition of tropical microalgae for scale-up production. *Biotechnol. Bioeng.,* 107 (2): 245–257.

Hemalatha, A., P.Karthikeyan, K. Girija, C. Saranya, P. Anantharaman and P. Sampathkumar 2014. Effect of nutrients on the growth and biochemical composition of the marine diatom *Chaetoceros simplex,* 5(1): 30-35.

Ichihara, K., A. Shibahara, K. Yamamoto and T. Nakayama 1996. An improved method for rapid analysis of the fatty acids of glycerolipids, *Lipids,* 31: 535–539.

Irshad Ahmad, I., Z. Fatma, S.S. Yazdani and S. Kumar 2013. DNA barcode and lipid analysis of new marine algae potential for biofuel. *Algal Research,* 2: 10–15.

Jalal, K.C.A., A.A. Shamshuddin, M.F. Rahman, N.Z. Nurazatul and M.Rozihan 2012. Growth and total carotenoid, chlorophyll aand chlorophyll b of tropical microalgae (*Isochrysis* sp.) in laboratory culture condition. *J. Biol. Sci.,* DOI: 3923/jbs 2012.

Ju, Z., L. Ding, Q. Zheng, Z. Wu and F. Zheng 2011. Diatoms as a Model System in Studying Lipid Biosynthesis regulation. *Intnl. J. Environ. Sci. Devl.,* 2(6).

Kim, J. D. and C. G. Lee 2005. Systemic optimization of microalgae for bioactive compound production. *Biotechnol. Bioproc. Eng*., 10: 418-424.

Lombardi, A.T. and P.J.Wangersky, PJ (1991). *Mar. Ecol. Prog. Ser*., **77**, 39 (1991).

Lopez-Elias, J.A., D. Voltolina, F. Enriquez-Ocana and G. Gallegos-Simental 2005. Indoor and outdoor mass production of the diatom *Chaetoceros muelleri* in a mexican commercial hatchery. *Aquacult. Eng.,* 33 (3): 181–191.

Meher, L.C., S.D. Vidya and S.N. Naik 2006.Technical aspects of biodiesel production by transesterification—a review. *Renew Sust Energy Rev*., 10:248–68.

Miao, X., R. Li and H. Yao 2009. Effective acid-catalyzed transesterification for biodiesel production. *Energy Convers. Manage.,* 50 (10): 2680–2684.

Mohan, N., M.G. Rajaram, A.B. Boopathy and R. Rengasamy 2012. Biomass and lipid production of marine diatom *Amphiprora paludosa* W. Smith at different nutrient concentrations. *J. Algal Biomass Utln*., 3(4): 52–59.

Renaud, S.M., L.V.Thin, G. ambrinidis and D.L.Parry 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture,* 211(1–4): 195–214.

Sheehan, J., T. Dunahay, J. Benemann and P. Roessler 1998. *A look back at U.S. department of energy's aquatic species program-biodiesel from algae*, NREL, Golden, Colorado, USA.

Sumper, M. and E. Brunner 2008. Silica biomineralisation in diatoms: the model organism *Thalassiosira pseudonana*. *Chembiochem.,* 9:1187–1194

Sing, S.F., A. Isdepsky, M.A. Borowitzka and N.R. Moheimani 2013).

Production of biofuels from microalgae. Mitig. Adapt. *Strategies Global Change,* 18:9227–9236.

Shalaby, E.A. 2011. Algal Biomass and Biodiesel Production. In: Stoytcheva, M. (Ed.), Biodiesel-Feedstocks and Processing Technologies. Intech, Croatia. pp. 111-458.

Subhramanyan, R. 1946. A systematic account of the marine plankton diatoms of the Madras coast, *Proc. Ind. Acad. Sci.,* 24B 85-197.

Takagi, M., K. Watanabe, K. Yamaberi and T. Yoshida 2000. Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of *Nannochloropsis* sp. UTEX LB1999. *Appl Microbiol .Biotechnol.,* 54: 112-117.

Thajuddin, N., A. Ilavarasi, E. Baldev, E. MubarakAli, N.S. Alharbi, A.Chinnathambi and S.A. Alharbi 2015. Stress induced lipids accumulation in naviculoid marine diatoms for bioenergy application. *International Journal of Biotechnology for Wellness Industries,* 2015, 4, 18-24.

Tomas, C.R. 1997. Identifying marine phytoplankton. Academic press, California, USA. pp.858.

Tran, H. L., J. S. Kwon, Z. H. Kim, Y. K. Oh and C. G. Lee 2010 Statistical optimization of culture media for growth and lipid production of Botryococcus braunii LB572. *Biotechnol. Bioproc. Eng.,* 15: 277-284.

Venkataraman, G. 1939. A systematic account of some south Indian diatoms, *Proc. Ind. Acad. Sci.,* 10**:** 293-368.

Vicente, G., L.F. Bautista, R. Rodriguez, J. Gutierrez, I. Sadaba, R.M.Ruiz-Vazquez, S. Torres-Martinez and V. Garre 2009. Biodiesel production from biomass of an oleaginous fungus, *Biochem. Eng. J.,* 48 : 22–27.

Garre, V. 2009. Biodiesel production from biomass of an oleaginous fungus, *Biochem. Eng. J.,* 48 : 22–27.

Wang, X.W., J.R. Liang, C.S. Luo, C.P. Chen and Y.H. Gao 2014. Biomass, total lipid production, and fatty acid composition of the marine diatom Chaetoceros muelleri in response to different CO2 levels. *Bioresource Technology* ,161: 124–130.

© Seaweed Research and Utilisation Association