Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), 12(X), 2024:
http://dx.doi.org/10.7770/safer-V12N1-art700

Impact of salt stress on growth and lipid content in oleaginous green microalgae

Impacto del estrés salino en el crecimiento y contenido lipídico en microalgas verdes oleaginosas

Jeetendra Singh[1*] and S.K. Soni[2]

[1]PhD Scholar, Department of Botany, Dayalbagh Educational Institute, Agra-5 (India)

[2]Associate Professor Department of Botany, Dayalbagh Educational Institute, Agra-5 (India)

E-mail: drsantendrasoni@gmail.com, jeetendrasingh60093@gmail.com

ABSTRACT

At present time, about 70% of energy is generated from fossil fuels and only about 30% is produced from renewable energy sources. The forecasted energy demand increases make evident that the conventional oil reserves that can be commercially exploited was vanished after 2050. Deteriorating Environmental condition demands sustainable renewable energy sources with least greenhouse gases emission. Third generation biofuel from microalgae can fulfill are demand of environmentally friendly biofuel with least GHG emission. In present research an affected has been made to enhance lipid content via saline stress. Lipid estimate showed a significant source in lipid. It may help to curb are dependency on fossil fuels. Fossil fuels are high energy sources, but these are Reduce day to day. These fuels high demand present in time but fossil stock is limited. The present work is a particularly important and unique work to cope with fossil fuel deficiency and greenhouse gases (GHGs). Oleaginous green microalgae are very efficient to overcome fossil fuel deficiency and environment related problem. In this work we will cultivate in-vitro three different microalgae which have significant amount of lipid. And second phase saline stress was employed to enhance lipid content in the selected microalgae.

Key Words Transesterification, Third generation Biofuels, Oleaginous, microalgae, Biodiesel

RESUMEN

En la actualidad, alrededor del 70% de la energía se genera a partir de combustibles fósiles y solo alrededor del 30% se produce a partir de fuentes de energía renovables. Los aumentos previstos en la demanda de energía hacen evidente que las reservas de petróleo convencional que se pueden explotar comercialmente se desvanecieron después de 2050. El deterioro de las condiciones ambientales exige fuentes de energía renovables sostenibles con la menor emisión de gases de efecto invernadero. El biocombustible de tercera generación a partir de microalgas puede satisfacer la demanda de biocombustible ecológico con la menor emisión de GEI. En la presente investigación se ha realizado un efecto para mejorar el contenido de lípidos a través del estrés salino. La estimación de lípidos mostró una fuente significativa de lípidos. Puede ayudar a frenar la dependencia de los combustibles fósiles. Los combustibles fósiles son fuentes de alta energía, pero estos se reducen día a día. Estos combustibles presentan una alta demanda en el tiempo pero el stock fósil es limitado. El presente trabajo es particularmente importante y único para hacer frente a la deficiencia de combustibles

fósiles y los gases de efecto invernadero (GEI). Las microalgas verdes oleaginosas son muy eficientes para superar la deficiencia de combustibles fósiles y los problemas relacionados con el medio ambiente. En este trabajo vamos a cultivar in vitro tres microalgas diferentes que tienen una cantidad significativa de lípidos. Y se empleó una segunda fase de estrés salino para mejorar el contenido de lípidos en las microalgas seleccionadas.
Palabras clave: Transesterificación, Biocombustibles de tercera generación, Oleaginosas, microalgas, Biodiesel

INTRODUCTION

Biodiesel is a biodegradable and renewable fuel which can be produced from a variety of feedstock and is currently being produced in many parts of the world. (Edith Martinez-Guerra et al. 2018). Biodiesel production may not completely replace the fossil fuel consumption, diminish the dependency on this conventional depleting sources. One of the major drawbacks of biodiesel is the feedstock availability for its production. However, the use of microalgae has demonstrated its capabilities of producing non-toxic, high-quality biodiesel.

Transesterification is simply the conversion of lipids in the feedstock into biodiesel by adding an ethanol and methanol are the most common acyl acceptors used for biodiesel production; while the catalyst can be acidic, basic, or enzymatic. (Ma F, Hanna MA et al. 1999).

Microorganisms that can be found in freshwater, wastewater, and marine water sources. Their characteristics are like those of other plants, however, microalgae are more efficient converting solar energy because of their simple cellular structure (Arun N, Singh DP et al. 2012).

They use sunlight for reducing CO_2 to biofuels, and they can accumulate significant amounts of triglycerides within their cells suitable for biofuel production through transesterification reaction (Dai YM, Chen et al. 2014).

The lipid accumulation in microalgal cells ranges from 25 to 75% of its dry weight (Boussiba S et al. 1987). Moreover, microalgae have the potential of fixing 1.83 tons of atmospheric CO_2 when producing one ton of algae biomass (Chisti Y. 2007). Also, microalgae are oil producing factories producing one hundred times more oil per acre than any other plants (Mubarak M, Shaija S, et al. 2015).

The rapid economic growth that took place in the second half of the 20th century caused a re-orientation in the manner of utilization of energy raw materials. A new model of the world economy has developed based on petroleum and natural gas, with a declining importance of hard coal (Ryan et al., 2006; Mata et al., 2010). However, the resources of those raw materials deplete fast, and their use causes several unfavorable effects, such as acid rains or global warming with the resultant climate changes (Demirbas, 2007; Somerville, 2007).

The dependence of the world economy on oil is such that speculations concerning the exhaustion of the raw material may result in a crisis in the world market. Such a phenomenon happened thrice in 1973, at the turn of 1980 and 1981 and 2008, when the price of oil soared to the level of 146.14 dollars per barrel (Huang et al., 2010). Apart from this, transport and the energy producing industry are the primary anthropogenic sources of greenhouse gas emissions in the European union that are responsible for more than 20 and 60%, respectively, of that emission (Mata et al., 2010).

In recent research microalgae such as Feedstock avoids these issues and presents several distinct advantages of not requiring agricultural or arable lands for production high photosynthetic efficiencies and biomass productivities (biomass doubled in less than one day), and one hundred times more lipids per acre of land [M. R Tredici, 2010]. Moreover, the main storage lipids in microalgae are neutral lipids (NLs) or triacylglycerols that can be esterified to FAMEs with the primary profiles of C16 and C18, proven to be the most suitable for biofuel production [S. Champagne Ge, P et al. 2017].

Energy crisis, global warming, and climate changes concern on the sustainability issues of fossil fuels utilization and energy supply. Biofuels as types of renewable, sustainable energy are recognized with the highest potential to satisfy the global energy demand.

Biofuels can be divided into three categories: First generation biofuels use edible feedstock such as soya beans, wheat, corn, rapeseed, oil crops, maize, sugarcane, and sugar beet, while second generation biofuels are derived from wastes and dedicated lignocellulosic feedstock such as switch grass (*Panicum virgatum*) and *Jatropha* (Zhu, et al. 2016). One of the major disadvantages of both first- and second-generation biofuels is that the cultivation of these food or nonfood crops as biofuel feedstock might compete for limited arable farmland, which should be utilized to cultivate crops as food feedstock, (Zhu et al. 2016).

Due to continuous and increasing combustion of fossil fuel the amount of greenhouse gas CO_2 has increased. As a results global warming and climate change are threatening ecological stability, food security and social welfare (Chisti, 2008; Christenson, 2011). The transportation and energy sector are the two major sources, responsible for the generation of 20% and 60% of greenhouse gases (GHG) and fossil fuels emissions, respectively, and it is expected that with the development of emerging economies the global consumption of energy will rise, and this will lead to more environmental damage (Stephens, et al.2011).

Lipids in the form of triacyl glycerides typically provide a storage function in the cell that enables microalgae to endure adverse environmental conditions (Giorgos and Elis, 2013; Kalpesh, et al. 2012). Studies have indicated that the lipid content of microalgae can be enhanced by changing the cultivation conditions and objecting them to diverse stress conditions (Pittman, et al. 2011; Devi and Venkta Mohan, 2014).

The major stress conditions applied to enhance lipid accumulation are temperature. Light intensity, pH, salinity, minerals salt and nutrients (Takagi, 2006; Ifeanyi, et al. 2011; Devi, et al. 2012).

Salinity stress can also lead to increment in the lipids content of microalgae due to its crucial role in causing changes in the fatty acid metabolism (Kalita, et al. 2011). Under high salinity stress, many organisms including microalgae alter their metabolism to adapt to the extreme environment (Kan, et al. 2012). The ability of microalgae to survive in saline environment under the influence of osmotic stress has received considerable attention which can also affect cell growth and lipid formation (Asulabh, et al. 2012).

Fluctuation in the salt content of the growth medium has also been found to alter the lipid composition of microalgae (Kalpesh, et al. 2012). As, algae are inhabitant of biotopes characterized by varying salinities, they have gained significance in salt tolerance studies domain any have served as model organisms for better understanding of salt acclimation in more complex physiological processes (Talebi, et al. 2013; Alkayal, et al. 2011).

Microalgae are being employed as potential generation vehicles to harness various useful products like biofuels, nutraceuticals, animal feeds and biomaterials etc. (Singh, et al., 2016).

MATERIAL AND METHODS

Microalgae and media composition: *Chlorella pyrenoidosa*, C*hlorella protothecoides* and *Scenedesmus obliquus* was procured from National Collection of Industrial Microorganisms (NCIM), Pune, India. Stock cultures of these algae was cultivated in BG11 medium at temperature ranges 32±2ºC under natural day light illumination in twelve 250 ml Erlenmeyer flasks, four for each alga. 150 ml freshly prepared BG11 medium was taken into each erlenmeyer flask, and each was inoculated with 20 % of inoculum i.e., 30 ml. Each liter of the BG11 medium contained $NaNO_3$-1.5 g, K_2HPO_4-0.04 g, $MgSO_4$ $7H_2O$-0.075 g, CaCl $22H_2O$-0.036 g, Citric acid-0.006 g, $NaCO_3$-0.02 g, H_3BO_3-0.00286 g, $MnCl_2$ $4H_2O$-0.00181 g, $ZnSO_4$ $7H_2O$-0.00022 g, Na_2MoO $42H_2O$-0.00039 g, $CuSO_4$ $5H_2O$-0.00008 g, Co $(NO_3)_2$ $6H_2O$-0.00005 g, $(NH_4)6Mo_7O_{24}$ $4H_2O$-0.003 g, Na_2EDTA-0.00001 g. The inoculums of known optical density (OD) at 680 nm was used to inoculate 1800 ml of freshly prepared BG11 medium. All the cultures were inoculated under aseptic conditions to avoid contamination. Illumination of 11 µmol m^{-2} s^{-1} at 32±2°C was provided to the culture for four days on an orbital shaker at 120 rpm.

These cultures were selected based on their lipid or fats. BG11 nutrient media was used to cultivate selected oleaginous green algae. Different saline concentrations of NaCl dose were prepared and applied to evaluate the impact of salt stress on lipid content of the selected algae. Different saline NaCl concentration was 0, 10, 25, 50, mM. All experiments were executed in triplicates. All the working solutions was inoculated by 3 % (v/v) of stock algal species cultures. The flasks was incubated under natural day light illumination at 11 µmol (photon)/m^2s-1 at 32±2ºC for 20 days with shaking at 120 rpm. 10 min. d^{-1}.

Culture conditions: The composition and quantity of lipids content are species-dependent and can be affected by external cultivation conditions, such as light intensity, temperature, carbondioxide, nutrient starvation, salinity stress, pH. All algal species are cultivated and maintained in natural conditions.

Biomass Growth Measurement: Optical density of growing micro algal culture was recorded by UV-Visible Spectrophotometer 2203 (SYSTRONICS) at 680 nm. Optical density of culture was recorded a regular basis at the interval of 4 days upto 20 days.

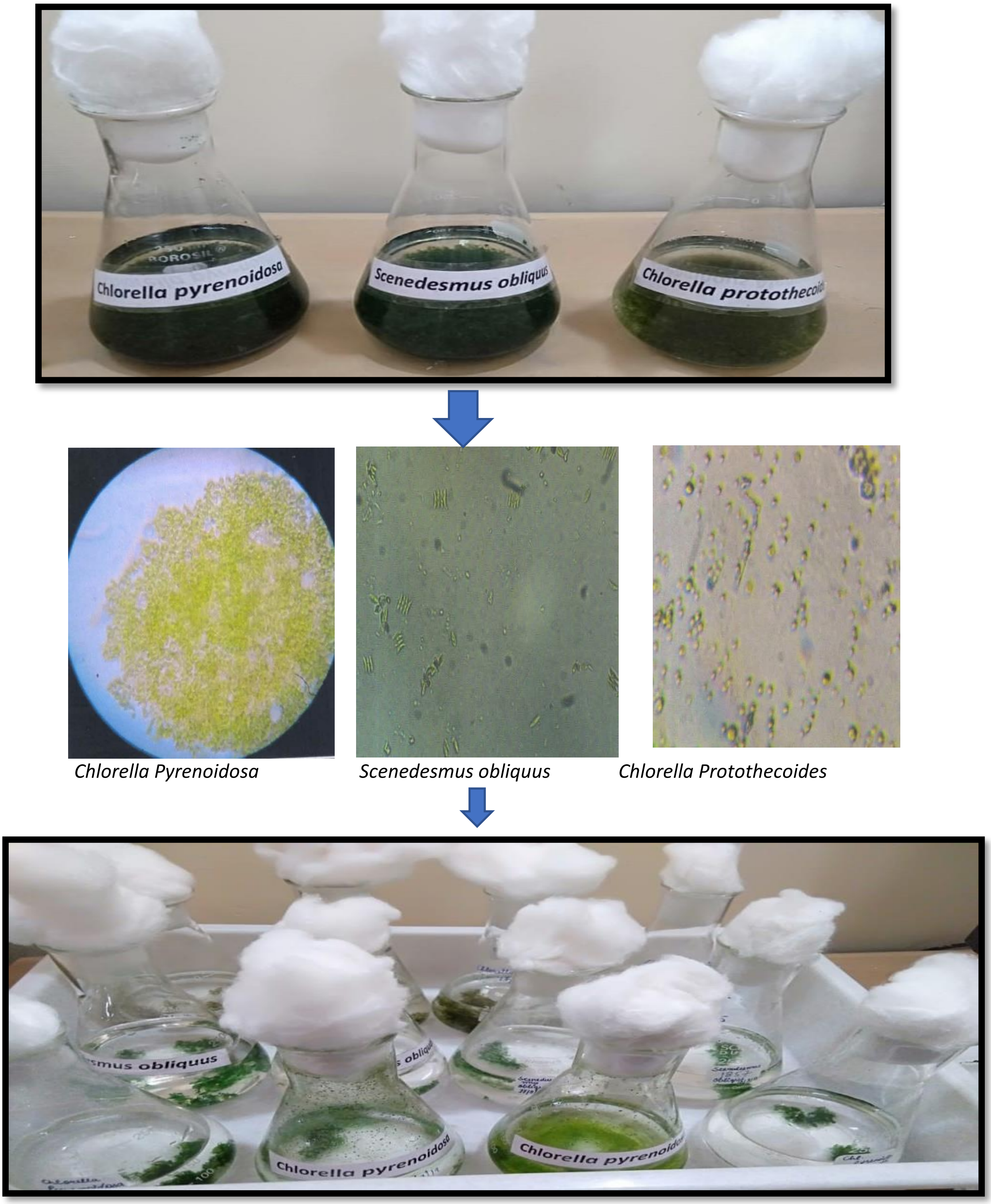
Measurement of lipid content (Folch et. al. 1957): Dried microalgae (40 mg) were taken in 5 ml of extraction solvent chloroform: methanol (2:1 v/v) The extract was filter via Whatman no.1 filter paper and 3 ml of 1% NaCl (1gm/100ml) was added to the filtrate. The resulting solution was transferred to a separating funnel to for separation. The lower organic phase containing the lipid components was collected in a small pre-weighed petri plate. It was kept overnight in a dessicator at room temperature in dark place. The beaker containing the dried extracts was reweighed and total lipids was estimated by subtracting the initial weight from the final weight.

RESULT AND DISCUSSION

Cultivation of Oleaginous green microalgae: There are three microalgae which have been elected for research work were *Chlorella pyrenoidosa*, *Chlorella protothecoides* and *Scendesmus obliquus* all were pure

Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), 12(X), 2024:
http://dx.doi.org/10.7770/safer-V12N1-art700

culture and procured from National Collection of Industrial Microorganism (NCIM), Pune, Maharashtra, Precured algae were sub-cultured in prescribed medium at temperature ranges 32±2°C under natural day light illumination in twelve 250 ml Erlenmeyer flasks, experiment was executed in triplicate.



Figures-1 Cultivation of green oleaginous microalgae

Sustainability, Agri, Food and Environmental Research, (ISSN: 0719-3726), 12(X), 2024:
http://dx.doi.org/10.7770/safer-V12N1-art700

Spectroscopy of microalgal cultures (Fig 3)

Chlorella pyrenoidosa, *Scendesmus obliquus* and *Chlorella protothecoides* were cultured in prescribed medium BG11 broth upto 20 days. Optical density was recorded at the interval of 4 days upto 20 days at 680nm via UV-Visible Double Beam Spectrophotometer 2203.

In *Chlorella pyrenoidosa* maximum O.D. was recorded in those culture stressed with 10mM, non-iodized NaCl, while least O.D recorded in cultures. Which stressed with 50mM non-iodized NaCl. However, optical density was maximum i.e, 0.706nm in control.

Results showed that saline stress inhibit growth rate of algae in some extent.

Spectroscopic access of *Scendesmus obliquus*

In *Scendesmus obliquus* maximum O.D. was recorded in those culture stressed with 10mM, non-iodized NaCl, while least O.D recorded in cultures. Which stressed with 50mM non-iodized NaCl. However, optical density was maximum i.e, 0.192nm in control. Results showed that saline stress inhibit growth rate of algae in some extent.

Spectroscopic access of *Chlorella protothecoides*

In *Chlorella protothecoides* maximum O.D. was recorded in those culture stressed with 10mM, non-iodized NaCl, while least O.D recorded in cultures. Which stressed with 50mM non-iodized NaCl.However, optical density was maximum i.e, 0.427nm in control. Results showed that saline stress inhibit growth rate of algae in some extent.

Lipid Estimation (Folch et al., 1957)

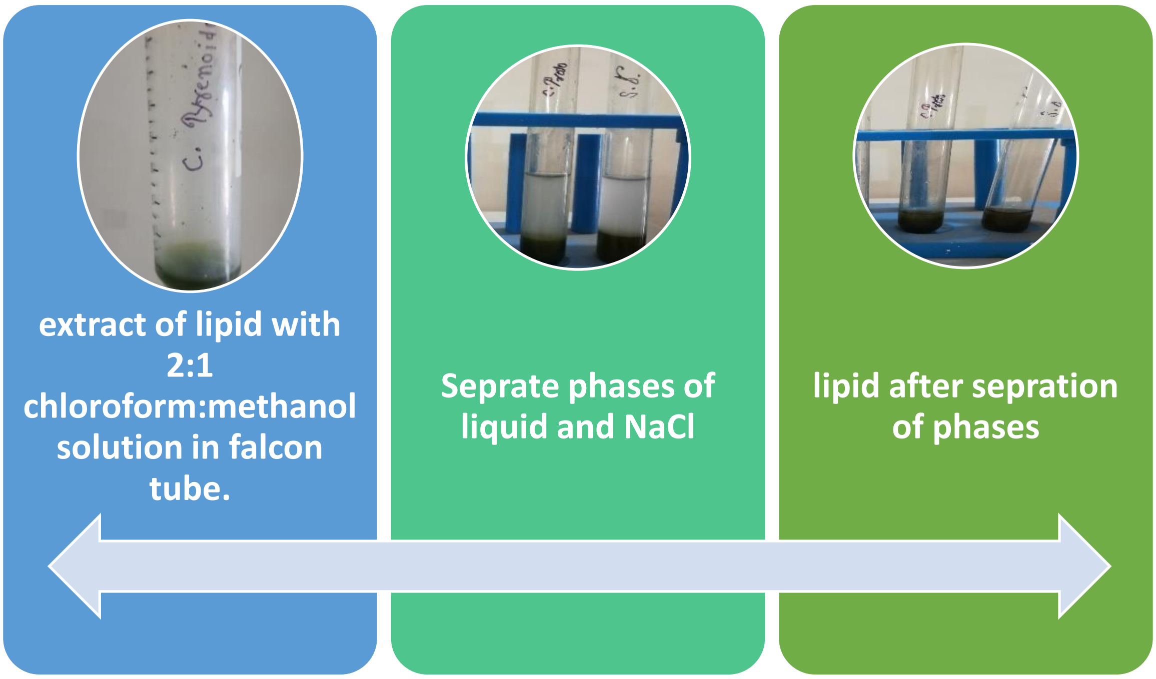
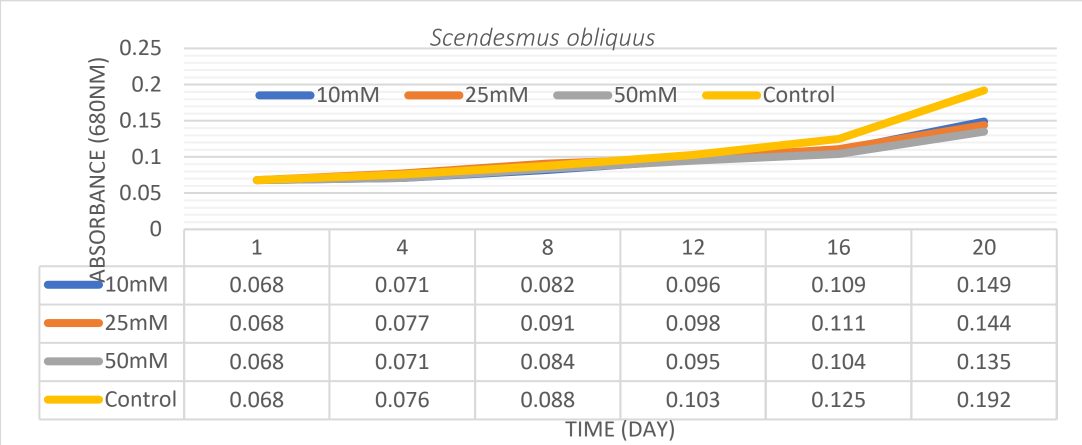
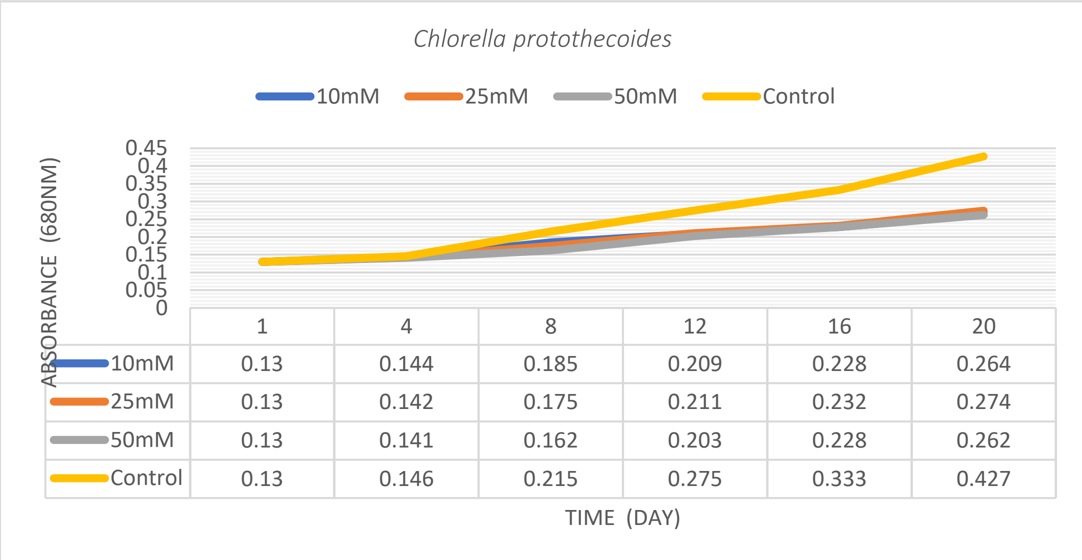


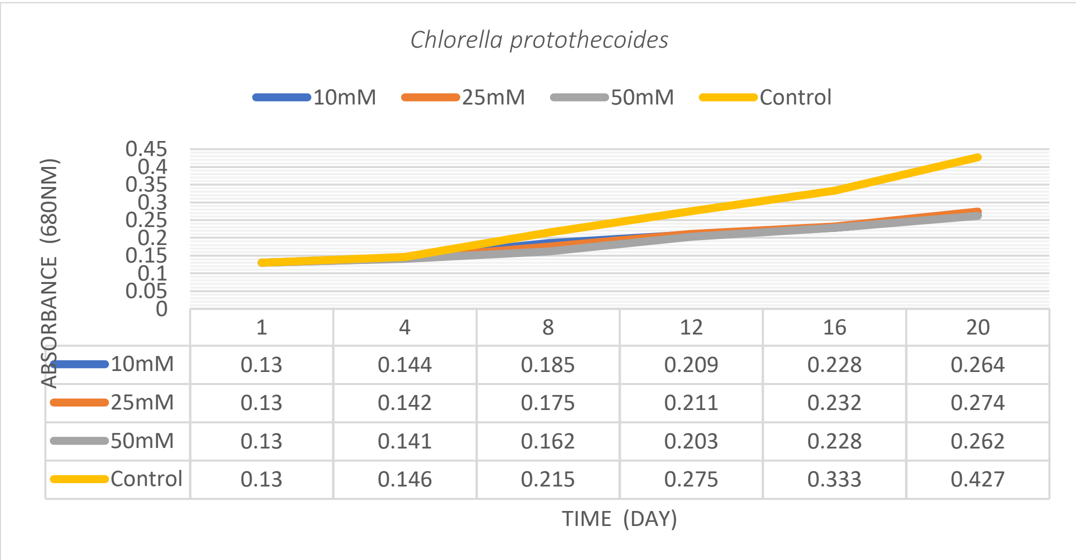
Figure-2 Lipid Estimation by Folch method



	1	4	8	12	16	20
10mM	0.068	0.071	0.082	0.096	0.109	0.149
25mM	0.068	0.077	0.091	0.098	0.111	0.144
50mM	0.068	0.071	0.084	0.095	0.104	0.135
Control	0.068	0.076	0.088	0.103	0.125	0.192



	1	4	8	12	16	20
10mM	0.13	0.144	0.185	0.209	0.228	0.264
25mM	0.13	0.142	0.175	0.211	0.232	0.274
50mM	0.13	0.141	0.162	0.203	0.228	0.262
Control	0.13	0.146	0.215	0.275	0.333	0.427


Chlorella protothecoides

	1	4	8	12	16	20
10mM	0.13	0.144	0.185	0.209	0.228	0.264
25mM	0.13	0.142	0.175	0.211	0.232	0.274
50mM	0.13	0.141	0.162	0.203	0.228	0.262
Control	0.13	0.146	0.215	0.275	0.333	0.427

Figure 3. Spectroscopy of microalgal cultures

A significant surge has been recorded in lipid content when algae were stressed with non-iodized NaCl. Maximum lipid content 28.5mg has been recorded in *Chlorella protothecoides* when stressed. while lipid content was 20mg when not stressed. In *Scendesmus obliquus* maximum lipid content 4.8 mg has been recorded when stressed. While lipid content was 9.6mg when not stressed. In *Chlorella pyrenoidosa* maximum lipid content 19.3 mg has been recorded when stressed. While lipid content was 8mg when not stressed (Fig. 4).

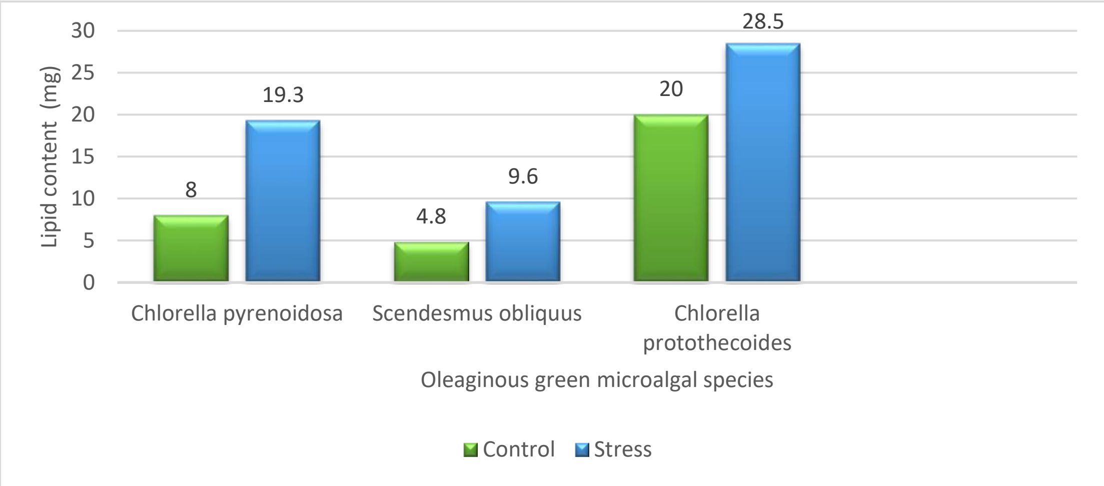


Fig . 4. Lipid content in Oleaginous green microalgae

CONCLUSION

Oleaginous green microalgae- *Chlorella pyrenoidosa*, *Chlorella protothecoides* and *Scendesmus obliquus* are potent for biofuel production. After maintained microalgal have been sub-cultured successfully in phycology lab, DEI Dayalbagh Agra. After experimentation of impact of saline stress, a significant stage in lipid content of all selected green microalgae has been observed. Although growth rate of microalgae is affected due to saline stress. Spectroscopic data has revealed slight depression in growth rate of microalgae.

Significance and prospects: At present time, about 70% of energy is generated from fossil fuels and only about 30% is produced from renewable energy sources. The forecasted energy demand increases make evident that the conventional oil reserves that can be commercially exploited was vanished after 2050. Deteriorating Environmental condition demands sustainable renewable energy sources with least greenhouse gases emission. Third generation biofuel from microalgae can fulfill are demand of environmentally friendly biofuel with least GHG emission. In present research an affected has been made to enhance lipid content via saline stress. Lipid estimate showed a significant source in lipid. It may help to curb are dependency on fossil fuels. Fossil fuels are high energy sources, but these are Reduce day to day. These fuels high demand present in time but fossil stock is limited. These sources are not renewable energy sources. And Oleaginous green microalgae are a high energy sources and high lipid content. These energy sources are a renewable and sustainable energy sources. Thus, this source is environment friendly biofuels. Strategies for cost reduction is producing microalgal biodiesel by using a biorefinery based production strategy.

Data availability

All data are available in Department of Botany, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh Agra-5.

conflict of interest

None

Author Contribution

Jeetendra singh in complete the work and writing of the paper and S.K. Soni provide proper guidance for this work.

Funding declaration

None

Acknowledgement

We have very thankful to director DEI for provide infrastructure and lab facilities

REFRENCES

Adams, C., Godfrey, V., Wahlen, B., Seefeldt, L., & Bugbee, B. (2013). Understanding precision nitrogen stress to optimize the growth and lipid content tradeoff in oleaginous green microalgae. *Bioresource Technology*, *131*, 188-194. https://doi.org/10.1016/j.biortech.2012.12.143

Alcántara, C., García-Encina, P. A., & Muñoz, R. (2013). Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process. *Chemical Engineering Journal*, *221*, 238-246. https://doi.org/10.1016/j.cej.2013.01.100

Al-Safaar, A. T., Al-Rubiaee, G. H., & Salman, S. K. (2016). Effect of pHCondition on the Growth and Lipid Content of Microalgae Chlorella vulgaris&Chroococcus minor. *Journal of Scientific & Engineering Research*, *7*(11), 1139.

Alyabyev, A., Loseva, N., Gordon, L., Andreyeva, I., Rachimova, G., Tribunskih, V., Ponomareva, A., & Kemp, R. (2007). undefined. *Thermochimica Acta*, *458*(1-2), 65-70. https://doi.org/10.1016/j.tca.2007.03.003

Amaro, H. M., Guedes, A. C., & Malcata, F. X. (2011). Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy*, *88*(10), 3402-3410. https://doi.org/10.1016/j.apenergy.2010.12.014

Angles, E., Jaouen, P., Pruvost, J., & Marchal, L. (2017). Wet lipid extraction from the microalga Nannochloropsis Sp.: Disruption, physiological effects, and solvent screening. *Algal Research*, *21*, 27-34. https://doi.org/10.1016/j.algal.2016.11.005

Anitha, S., Shah, A., & Ali, B. (2018). Modulation of lipid productivity under nitrogen, salinity, and temperature stress in microalgae Sp. *Journal of Environmental Biology*, *39*(5), 625-632. https://doi.org/10.22438/jeb/39/5/mrn-761

Annamalai, J., Shanmugam, J., & Nallamuthu, T. (2016). Salt stress enhancing the production of phytochemicals in *Chlorella vulgaris* and *Chlamydomonas reinhardtii*. *Journal of Algal Biomass Utilization*, *7*(1), 37-44.

Arun, N., & Singh, D. P. (2012). Microalgae: the future fuel. *J Algal Biomass Utln*, *3*(1), 46-54.

Asulabh, K. S., Supriya, G., & Ramachandra, T.V. (2012). Effect of salinity concentrations on growth rate and lipid concentration in Microcystis sp., Chlorococcum sp. and Chaetoceros sp. In *National Conference on Conservation and Management of Wetland Ecosystems. School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala*.

Atikij, T., Syaputri, Y., Iwahashi, H., Praneenararat, T., Sirisattha, S., Kageyama, H., & Waditee-Sirisattha, R. (2019). Enhanced lipid production and molecular dynamics under salinity stress in green Microalga chlamydomonas reinhardtii (137C). *Marine Drugs*, *17*(8), 484. https://doi.org/10.3390/md17080484

Boussiba, S., Vonshak, A., Cohen, Z., Avissar, Y., & Richmond, A. (1987). Lipid and biomass production by the halotolerant microalga Nannochloropsis Salina. *Biomass*, *12*(1), 37-47. https://doi.org/10.1016/0144-4565(87)90006-0

Cabanelas, I. T., van der Zwart, M., Kleinegris, D. M., Wijffels, R. H., & Barbosa, M. J. (2016). Sorting cells of the microalga Chlorococcum littorale with increased triacylglycerol productivity. *Biotechnology for Biofuels*, *9*(1). https://doi.org/10.1186/s13068-016-0595-x

Cañavate, J. P., Armada, I., & Hachero-Cruzado, I. (2017). Aspects of phosphorus physiology associated with phosphate-induced polar lipid remodelling in marine microalgae. *Journal of Plant Physiology*, *214*, 28-38. https://doi.org/10.1016/j.jplph.2017.03.019

Chen, B., Wan, C., Mehmood, M. A., Chang, J., Bai, F., & Zhao, X. (2017). Manipulating environmental stresses and stress tolerance of microalgae for enhanced production of lipids and value-added products–A review. *Bioresource Technology*, *244*, 1198-1206. https://doi.org/10.1016/j.biortech.2017.05.170

Chen, C., Yeh, K., Aisyah, R., Lee, D., & Chang, J. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresource Technology*, *102*(1), 71-81. https://doi.org/10.1016/j.biortech.2010.06.159

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, *25*(3), 294-306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Chisti, Y. (2008). Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology*, *26*(3), 126-131. https://doi.org/10.1016/j.tibtech.2007.12.002

Christenson, L., & Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances*, *29*(6), 686-702. https://doi.org/10.1016/j.biotechadv.2011.05.015

D'Alessandro, E. B., & Antoniosi Filho, N. R. (2016). Concepts and studies on lipid and pigments of microalgae: A review. *Renewable and Sustainable Energy Reviews*, *58*, 832-841. https://doi.org/10.1016/j.rser.2015.12.162

Dai, Y., Chen, K., & Chen, C. (2014). Study of the microwave lipid extraction from microalgae for biodiesel production. *Chemical Engineering Journal*, *250*, 267-273. https://doi.org/10.1016/j.cej.2014.04.031

DEMIRBAS, A. (2007). Progress and latest trends in biofuels. *Progress in Energy and Combustion Science*, *33*(1), 1-18. https://doi.org/10.1016/j.pecs.2006.06.001

Dong, T., Knoshaug, E. P., Pienkos, P. T., & Laurens, L. M. (2016). Lipid recovery from wet oleaginous microbial biomass for biofuel production: A critical review. *Applied Energy*, *177*, 879-895. https://doi.org/10.1016/j.apenergy.2016.06.002

Dutta, S., Neto, F., & Coelho, M. C. (2016). Microalgae biofuels: A comparative study on techno-economic analysis & life-cycle assessment. *Algal Research*, *20*, 44-52. https://doi.org/10.1016/j.algal.2016.09.018

Ebrahimi, E., & Salarzadeh, A. (2016). The effect of temperature and salinity on the growth of Skeletonema costatum and chlorella capsulata in vitro. *International Journal of Life Sciences*, *10*(1), 40-44. https://doi.org/10.3126/ijls.v10i1.14508

Farooq, W., Mishra, S. K., Moon, M., Suh, W. I., Shrivastav, A., Kumar, K., Kwon, J. H., Park, M. S., & Yang, J. (2016). Energy efficient process for microalgae cell disruption for oil recovery using triiodide resin. *Algal Research*, *13*, 102-108. https://doi.org/10.1016/j.algal.2015.11.007

Fortier, M. P., Roberts, G. W., Stagg-Williams, S. M., & Sturm, B. S. (2017). Determination of the life cycle climate change impacts of land use and albedo change in algal biofuel production. *Algal Research*, *28*, 270-281. https://doi.org/10.1016/j.algal.2017.06.009

Gao, B., Huang, L., Wang, F., Chen, A., & Zhang, C. (2019). Bilateral and simultaneous accumulation of lipid and biomass in the novel oleaginous green microalga Tetradesmus bernardii under mixotrophic growth. *Algal Research*, *37*, 64-73. https://doi.org/10.1016/j.algal.2018.11.012

Ge, S., & Champagne, P. (2016). Nutrient removal, microalgal biomass growth, harvesting and lipid yield in response to centrate wastewater loadings. *Water Research*, *88*, 604-612. https://doi.org/10.1016/j.watres.2015.10.054

Ge, S., & Champagne, P. (2017). Cultivation of the marine Macroalgae *Chaetomorpha linum* in municipal wastewater for nutrient recovery and biomass production. *Environmental Science & Technology*, *51*(6), 3558-3566. https://doi.org/10.1021/acs.est.6b06039

Gorain, P. C., Bagchi, S. K., & Mallick, N. (2013). Effects of calcium, magnesium, and sodium chloride in enhancing lipid accumulation in two green microalgae. *Environmental Technology*, *34*(13-14), 1887-1894. https://doi.org/10.1080/09593330.2013.812668

Götz, M., Lefebvre, J., Mörs, F., McDaniel Koch, A., Graf, F., Bajohr, S., Reimert, R., & Kolb, T. (2016). Renewable power-to-Gas: A technological and economic review. *Renewable Energy*, *85*, 1371-1390. https://doi.org/10.1016/j.renene.2015.07.066

Gude, V. G., & Martinez-Guerra, E. (2017). Green chemistry with process intensification for sustainable biodiesel production. *Environmental Chemistry Letters*, *16*(2), 327-341. https://doi.org/10.1007/s10311-017-0680-9

Guihéneuf, F., Fouqueray, M., Mimouni, V., Ulmann, L., Jacquette, B., & Tremblin, G. (2010). Effect of UV stress on the fatty acid and lipid class composition in two marine microalgae Pavlova lutheri (Pavlovophyceae) and Odontella aurita (Bacillariophyceae). *Journal of Applied Phycology*, *22*(5), 629-638. https://doi.org/10.1007/s10811-010-9503-0

Hassan, F. M. (2013). Stimulation of biodiesel production from two algae: Chlorella vulgarisBerjerinckandNitzschiapalea (Kutz.) Smith, and study some growth parameters under different light intensity. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *6*(2), 31-42. https://doi.org/10.9790/2402-0623142

Hiremath, S., & Mathad, P. (2010). Impact of salinity on the physiological and biochemical traits of Chlorella vulgaris Beijerinck. *J Algal Biomass Utln*, *1*(2), 51-59.

Ho, S., Chen, W., & Chang, J. (2010). Scenedesmus obliquus CNW-N as a potential candidate for CO2 mitigation and biodiesel production. *Bioresource Technology*, *101*(22), 8725-8730. https://doi.org/10.1016/j.biortech.2010.06.112

Hu, W., Peng, C., Luo, W., Lv, M., Li, X., Li, D., Huang, Q., & Fan, C. (2010). Graphene-based antibacterial paper. *ACS Nano*, *4*(7), 4317-4323. https://doi.org/10.1021/nn101097v

Huang, G., Chen, F., Wei, D., Zhang, X., & Chen, G. (2010). Biodiesel production by microalgal biotechnology. *Applied Energy*, *87*(1), 38-46. https://doi.org/10.1016/j.apenergy.2009.06.016

Jayanta, T., Chandra, K. M., & Chandra, G. B. (2012). Growth, total lipid content and fatty acid profile of a native strain of the freshwater oleaginous microalgae Ankistrodesmus falcatus (Ralf) grown under salt stress condition. *International Research Journal of Biological Sciences*, *1*(8), 27-35. DOI:10.1007/s12210-016-0528-4

Johnson, T. J., Halfmann, C., Zahler, J. D., Zhou, R., & Gibbons, W. R. (2016). Increasing the tolerance of filamentous cyanobacteria to next-generation biofuels via directed evolution. *Algal Research*, *18*, 250-256. https://doi.org/10.1016/j.algal.2016.06.023

Khan, S. A., Rashmi, Hussain, M. Z., Prasad, S., & Banerjee, U. (2009). Prospects of biodiesel production from microalgae in India. *Renewable and Sustainable Energy Reviews*, *13*(9), 2361-2372. https://doi.org/10.1016/j.rser.2009.04.005

Khozin-Goldberg, I., Bigogno, C., Shrestha, P., & Cohen, Z. (2002). Nitrogen starvation induces the accumulation of arachidonic acid in the freshwater green alga parietochloris incisa (Trebuxiophyceae)one. *Journal of Phycology*, *38*(5), 991-994. https://doi.org/10.1046/j.1529-8817.2002.01160.x

Kirrolia, A., Bishnoi, N. R., & Singh, N. (2011). Salinity as a factor affecting the physiological and biochemical traits of Scenedesmus quadricauda. *Journal of Algal Biomass Utilization*, *2*(4), 28-34. DOI:10.3390/biom10111515

Knoshaug, E. P., Dong, T., Spiller, R., Nagle, N., & Pienkos, P. T. (2018). Pretreatment and fermentation of salt-water grown algal biomass as a feedstock for biofuels and high value biochemicals. *Algal Research*, *36*, 239-248. https://doi.org/10.1016/j.algal.2018.10.024

Knothe, G., Sharp, C. A., & Ryan, T. W. (2005). Exhaust emissions of biodiesel, Petrodiesel, neat methyl esters, and alkanes in a recent technology engine. *Energy & Fuels*, *20*(1), 403-408. https://doi.org/10.1021/ef0502711

Knutson, C. M., McLaughlin, E. M., & Barney, B. M. (2018). Effect of temperature control on green algae grown under continuous culture. *Algal Research*, *35*, 301-308. https://doi.org/10.1016/j.algal.2018.08.020

Kostas, E. T., White, D. A., & Cook, D. J. (2017). Development of a bio-refinery process to produce speciality chemical, biofuel, and bioactive compounds from laminaria digitata. *Algal Research*, *28*, 211-219. https://doi.org/10.1016/j.algal.2017.10.022

Kwak, H. S., Kim, J. Y., Woo, H. M., Jin, E., Min, B. K., & Sim, S. J. (2016). Synergistic effect of multiple stress conditions for improving microalgal lipid production. *Algal Research*, *19*, 215-224. https://doi.org/10.1016/j.algal.2016.09.003

Li, J., Li, C., Lan, C. Q., & Liao, D. (2018). Effects of sodium bicarbonate on cell growth, lipid accumulation, and morphology of chlorella vulgaris. *Microbial Cell Factories*, *17*(1). https://doi.org/10.1186/s12934-018-0953-4

Liang, Y., Sun, M., Tian, C., Cao, C., & Li, Z. (2014). undefined. *Botanica Marina*, *57*(6), 469-476. https://doi.org/10.1515/bot-2014-0037

Ma, F., & Hanna, M. A. (1999). Biodiesel production: A review1Journal series #12109, agricultural research division, Institute of agriculture and natural resources, University of Nebraska–lincoln.1. *Bioresource Technology*, *70*(1), 1-15. https://doi.org/10.1016/s0960-8524(99)00025-5

Maity, J. P., Bundschuh, J., Chen, C., & Bhattacharya, P. (2014). Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. *Energy*, *78*, 104-113. https://doi.org/10.1016/j.energy.2014.04.003

Markou, G., & Nerantzis, E. (2013). Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. *Biotechnology Advances*, *31*(8), 1532-1542. https://doi.org/10.1016/j.biotechadv.2013.07.011

Medipally, S. R., Yusoff, F. M., Banerjee, S., & Shariff, M. (2015). Microalgae as sustainable renewable energy feedstock for Biofuel production. *BioMed Research International*, *2015*, 1-13. https://doi.org/10.1155/2015/519513

Mubarak, M., Shaija, A., & Suchithra, T. (2015). A review on the extraction of lipid from microalgae for biodiesel production. *Algal Research*, *7*, 117-123. https://doi.org/10.1016/j.algal.2014.10.008

Newby, D. T., Mathews, T. J., Pate, R. C., Huesemann, M. H., Lane, T. W., Wahlen, B. D., Mandal, S., Engler, R. K., Feris, K. P., & Shurin, J. B. (2016). Assessing the potential of polyculture to accelerate algal biofuel production. *Algal Research*, *19*, 264-277. https://doi.org/10.1016/j.algal.2016.09.004

Omirou, M., Tzovenis, I., Charalampous, P., Tsaousis, P., Polycarpou, P., Chantzistrountsiou, X., Economou-Amilli, A., & Ioannides, I. M. (2018). Development of marine multi-algae cultures for biodiesel production. *Algal Research*, *33*, 462-469. https://doi.org/10.1016/j.algal.2018.06.025

Pal, D., Khozin-Goldberg, I., Cohen, Z., & Boussiba, S. (2011). The effect of light, salinity, and nitrogen availability on lipid production by Nannochloropsis Sp. *Applied Microbiology and Biotechnology*, *90*(4), 1429-1441. https://doi.org/10.1007/s00253-011-3170-1

Pal, D., Khozin-Goldberg, I., Cohen, Z., & Boussiba, S. (2011). The effect of light, salinity, and nitrogen availability on lipid production by Nannochloropsis Sp. *Applied Microbiology and Biotechnology*, *90*(4), 1429-1441. https://doi.org/10.1007/s00253-011-3170-1

Pittman, J. K., Dean, A. P., & Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology*, *102*(1), 17-25. https://doi.org/10.1016/j.biortech.2010.06.035

Prathima Devi, M., Swamy, Y., & Venkata Mohan, S. (2013). Nutritional mode influences lipid accumulation in microalgae with the function of carbon sequestration and nutrient supplementation. *Bioresource Technology*, *142*, 278-286. https://doi.org/10.1016/j.biortech.2013.05.001

Qiu, R., Gao, S., Lopez, P. A., & Ogden, K. L. (2017). Effects of pH on cell growth, lipid production and CO2 addition of microalgae chlorella sorokiniana. *Algal Research*, *28*, 192-199. https://doi.org/10.1016/j.algal.2017.11.004

Ratnapuram, H. P., Vutukuru, S., & Yadavalli, R. (2018). Mixotrophic transition induced lipid productivity in chlorella pyrenoidosa under stress conditions for biodiesel production. *Heliyon*, *4*(1), e00496. https://doi.org/10.1016/j.heliyon.2017.e00496

Rawat, I., Ranjith Kumar, R., Mutanda, T., & Bux, F. (2013). Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, *103*, 444-467. https://doi.org/10.1016/j.apenergy.2012.10.004

Salama, E., Kim, H., Abou-Shanab, R. A., Ji, M., Oh, Y., Kim, S., & Jeon, B. (2013). Biomass, lipid content, and fatty acid composition of freshwater chlamydomonas mexicana and Scenedesmus obliquus grown under salt stress. *Bioprocess and Biosystems Engineering*, *36*(6), 827-833. https://doi.org/10.1007/s00449-013-0919-1

Sarayloo, E., Tardu, M., Unlu, Y. S., Simsek, S., Cevahir, G., Erkey, C., & Kavakli, I. H. (2017). Understanding lipid metabolism in high-lipid-producing chlorella vulgaris mutants at the genome-wide level. *Algal Research*, *28*, 244-252. https://doi.org/10.1016/j.algal.2017.11.009

Sforza, E., Simionato, D., Giacometti, G. M., Bertucco, A., & Morosinotto, T. (2012). Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in Photobioreactors. *PLoS ONE*, *7*(6), e38975. https://doi.org/10.1371/journal.pone.0038975

Sharma, K. K., Schuhmann, H., & Schenk, P. M. (2012). High lipid induction in Microalgae for biodiesel production. *Energies*, *5*(5), 1532-1553. https://doi.org/10.3390/en5051532

Shi, K., Gao, Z., Shi, T., Song, P., Ren, L., Huang, H., & Ji, X. (2017). Reactive oxygen species-mediated cellular stress response and lipid accumulation in oleaginous microorganisms: The state of the art and future perspectives. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00793

Shi, R., Handler, R. M., & Shonnard, D. R. (2019). Life cycle assessment of novel technologies for algae harvesting and oil extraction in the renewable diesel pathway. *Algal Research*, *37*, 248-259. https://doi.org/10.1016/j.algal.2018.12.005

Singh, P., Guldhe, A., Kumari, S., Rawat, I., & Bux, F. (2015). Investigation of combined effect of nitrogen, phosphorus, and iron on lipid productivity of microalgae Ankistrodesmus falcatus KJ671624 using response surface methodology. *Biochemical Engineering Journal*, *94*, 22-29. https://doi.org/10.1016/j.bej.2014.10.019

Solovchenko, A. E. (2012). Physiological role of neutral lipid accumulation in eukaryotic microalgae under stresses. *Russian Journal of Plant Physiology*, *59*(2), 167-176. https://doi.org/10.1134/s1021443712020161

Somerville, C. (2007). Biofuels. *Current Biology*, *17*(4), R115-R119. https://doi.org/10.1016/j.cub.2007.01.010

Srivastava, G., Nishchal, & Goud, V. V. (2017). Salinity induced lipid production in microalgae and cluster analysis (ICCB 16-BR_047). *Bioresource Technology*, *242*, 244-252. https://doi.org/10.1016/j.biortech.2017.03.175

Stephens, E., Ross, I. L., King, Z., Mussgnug, J. H., Kruse, O., Posten, C., Borowitzka, M. A., & Hankamer, B. (2010). An economic and technical evaluation of microalgal biofuels. *Nature Biotechnology*, *28*(2), 126-128. https://doi.org/10.1038/nbt0210-126

Stephens, G. L., O'Brien, D., Webster, P. J., Pilewski, P., Kato, S., & Li, J. (2015). The albedo of earth. *Reviews of Geophysics*, *53*(1), 141-163. https://doi.org/10.1002/2014rg000449

Sun, X., Ren, L., Zhao, Q., Ji, X., & Huang, H. (2018). Microalgae to produce lipid and carotenoids: A review with focus on stress regulation and adaptation. *Biotechnology for Biofuels*, *11*(1). https://doi.org/10.1186/s13068-018-1275-9

Takagi, M., Karseno, & Yoshida, T. (2006). Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae Dunaliella cells. *Journal of Bioscience and Bioengineering*, *101*(3), 223-226. https://doi.org/10.1263/jbb.101.223

Talebi, A. F., Tabatabaei, M., & Chisti, Y. (2014). BiodieselAnalyzer: A user-friendly software for predicting the properties of prospective biodiesel. *Biofuel Research Journal*, 55-57. https://doi.org/10.18331/brj2015.1.2.4

Talukdar, J., Kalita, M. C., & Goswami, B. C. (2012). Effects of Salinity on Growth and Total Lipid Content of the Biofuel Potential Microalga Ankistro-desmus falcatus (Corda) Ralfs.

Teichner, E. M. (2015). Stress-induced production of lipids in oleaginous microalgae for biofuel optimization. https://doi.org/10.7287/peerj.preprints.1276v1

Teronpi, H., & Baruah, P. P. Salinity stress on growth and lipid accumulation in Anabaena circinalis: a preliminary study. DOI: 10.1007/s00284-019-01860-z

Torres, S., Acien, G., García-Cuadra, F., & Navia, R. (2017). Direct transesterification of microalgae biomass and biodiesel refining with vacuum distillation. *Algal Research*, *28*, 30-38. https://doi.org/10.1016/j.algal.2017.10.001

Tredici, M. R. (2010). Photobiology of microalgae mass cultures: Understanding the tools for the next Green Revolution. *Biofuels*, *1*(1), 143-162. https://doi.org/10.4155/bfs.09.10

Venkata Mohan, S., & Devi, M. P. (2014). Salinity stress induced lipid synthesis to harness biodiesel during dual mode cultivation of mixotrophic microalgae. *Bioresource Technology*, *165*, 288-294. https://doi.org/10.1016/j.biortech.2014.02.103

Venkata Mohan, S., & Devi, M. P. (2014). Salinity stress induced lipid synthesis to harness biodiesel during dual mode cultivation of mixotrophic microalgae. *Bioresource Technology*, *165*, 288-294. https://doi.org/10.1016/j.biortech.2014.02.103

Venkata Subhash, G., Rohit, M., Devi, M. P., Swamy, Y., & Venkata Mohan, S. (2014). Temperature induced stress influence on biodiesel productivity during mixotrophic microalgae cultivation with wastewater. *Bioresource Technology*, *169*, 789-793. https://doi.org/10.1016/j.biortech.2014.07.019

Vitova, M., Bisova, K., Kawano, S., & Zachleder, V. (2015). Accumulation of energy reserves in algae: From cell cycles to biotechnological applications. *Biotechnology Advances*, *33*(6), 1204-1218. https://doi.org/10.1016/j.biotechadv.2015.04.012

Wang, S. T., Pan, Y. Y., Liu, C. C., Chuang, L. T., & Chen, C. N. N. (2011). Characterization of a green microalga UTEX 2219-4: effects of photosynthesis and osmotic stress on oil body formation. *Botanical Studies*, *52*(3).

Welter, C., Schwenk, J., Kanani, B., Van Blargan, J., & Belovich, J. M. (2013). Minimal medium for optimal growth and lipid production of the microalgae*Scenedesmus dimorphus*. *Environmental Progress & Sustainable Energy*, *32*(4), 937-945. https://doi.org/10.1002/ep.11835

WU, Y., LI, Q., JIN, R., CHEN, W., LIU, X., KONG, F., KE, Y., SHI, H., & YUAN, J. (2019). Effect of low-nitrogen stress on photosynthesis and chlorophyll fluorescence characteristics of maize cultivars with different low-nitrogen tolerances. *Journal of Integrative Agriculture*, *18*(6), 1246-1256. https://doi.org/10.1016/s2095-3119(18)62030-1

Xu, H., Lee, U., Coleman, A. M., Wigmosta, M. S., & Wang, M. (2019). Assessment of algal biofuel resource potential in the United States with consideration of regional water stress. *Algal Research*, *37*, 30-39. https://doi.org/10.1016/j.algal.2018.11.002

Yadavalli, R., Peasari, J. R., Mamindla, P., Praveenkumar, Mounika, S., & Ganugapati, J. (2018). Phytochemical screening and in silico studies of flavonoids from chlorella pyrenoidosa. *Informatics in Medicine Unlocked*, *10*, 89-99. https://doi.org/10.1016/j.imu.2017.12.009

Yang, L., Tan, X., Si, B., Zhao, F., Chu, H., Zhou, X., & Zhang, Y. (2018). Nutrients recycling and energy evaluation in a closed microalgal biofuel production system. *Algal Research*, *33*, 399-405. https://doi.org/10.1016/j.algal.2018.06.009

Yasmin Anum Mohd Yusof, (2011). Fatty acids composition of microalgae chlorella vulgaris can be modulated by varying carbon dioxide concentration in outdoor culture. *African Journal of Biotechnology*, *10*(62). https://doi.org/10.5897/ajb11.1602

Zhu, L. (2015). Biorefinery as a promising approach to promote microalgae industry: An innovative framework. *Renewable and Sustainable Energy Reviews*, *41*, 1376-1384. https://doi.org/10.1016/j.rser.2014.09.040

Zhu, L. D., Li, Z. H., & Hiltunen, E. (2016). Strategies for lipid production improvement in Microalgae as a biodiesel feedstock. *BioMed Research International*, *2016*, 1-8. https://doi.org/10.1155/2016/8792548

Zienkiewicz, K., Du, Z., Ma, W., Vollheyde, K., & Benning, C. (2016). Stress-induced neutral lipid biosynthesis in microalgae — Molecular, cellular, and physiological insights. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1861*(9), 1269-1281. https://doi.org/10.1016/j.bbalip.2016.02.008

Received: 09th May 2023; Accepted: 11th Jule 2023; First distribution: 19th September 2023.