Biocatalysis and Agricultural Biotechnology 17 (2019) 583–588

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Microalgae as rich source of polyunsaturated fatty acids

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ARTICLE INFO

Keywords:
Biodiesel
Fatty acid
Lipid
Microalgae
Nutrient stress
PUFA

ABSTRACT

Microalgae are rich source of poly unsaturated fatty acids and therefore, extraction and purification of poly unsaturated fatty acids from algal strains are emerging steeply to nourish the nutritional needs of the population. Recently, various cost-effective and efficient approaches are being explored to increase the poly unsaturated fatty acids yield in algae. In this juncture, this article attempted to connect extraction of lipid using different methods, and accumulation of lipid under various stress conditions, and accumulation of poly unsaturated fatty acids under various stress conditions. Initially, extraction of lipid from various algal strains using different techniques was discussed in detail. Then, accumulation of lipid in microalgae under the influence of various stimuli has been critically flagged, in order to enable researchers to choose an optimal lipid induction condition for food and fuel production. Later, impact of various factors or culture condition on the poly unsaturated fatty acids accumulation in algae has been extensively assessed and presented in the perspective of increasing its production for food supplement. Finally, recent literature pertaining to the lipid and poly unsaturated fatty acids production in microalgae is reviewed, and summarized.

1. Introduction

Generally, algae are extremely diverse group of eukaryotic and prokaryotic photosynthetic organisms, which possess diverse physiological, morphological and genetic traits to confer the capability to yield various bioactive compounds. Chlorophyta is one of the largest phyla with different species and wide geographical distributions (Kumar et al., 2017). These microscopic organisms show different morphologies with approximately 3–10 μm (length or diameter) and support approximately 40% of global photosynthesis (Moreno-Garrido, 2008). Photosynthetic ability and ubiquitous nature of microalgae made them a rich source of primary productivity in a wide range of habitats including fresh-, sea-, and brackish water (Mathimani and Mallick, 2018). In this energy deficient era, there is an increasing demand of alternative fuel to substitute fossil fuel for meeting commercial viability (Mathimani et al., 2017a; Mathimani and Nair, 2016; Saravanan et al., 2018; Sayeda et al., 2015). In the past decade, focus has been shifted to the production of third generation biofuels from microalgae (Biller et al. (2013; Mathimani et al., 2018a), and microalgae have been found to be a competent source of biofuels production due to certain advantages such as increased lipid, and biomass productivity, and preferable fatty acid profile (Mathimani et al., 2017b, 2015), high photosynthetic rate, requirement of low culturing land, and tolerance to various environmental conditions (Griffiths et al., 2012).

In addition to the biofuel production, microalgae have been demonstrated as a potential source of phenolic compounds and other antioxidant compounds (Morowvat and Ghasemi, 2016) and the bioactive compounds extracted from microalgae exhibit pharmaceutical, healthcare and food industries applications (Sahu et al., 2013) and further, these non-energy products have high commercial value (Mathimani and Pugazhendhi, 2018). Microalgal metabolism depends on the external conditions and therefore slight changes in external environment or culture conditions stimulate the production of various commercially important metabolites. Microalgal lipids are considered as valued constituents in aquaculture feeds (Axelsson and Gentili, 2014). Among value added products produced from microalgae, production of polyunsaturated fatty acids (PUFA) or long-chain fatty acids are broadly known for their beneficiary effects on human health

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https://doi.org/10.1016/j.bcab.2019.01.017
Received 27 November 2018; Received in revised form 28 December 2018; Accepted 9 January 2019
Available online 10 January 2019
1878-8181/

(Guihéneuf and Stengel, 2013) and the nutritional value of microalgae is mainly related to their essential fatty acids contents (Liang et al., 2006). Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) are nutritionally significant PUFA's produced in significant amounts by the microalgal species, which increased over the trophic levels in the marine food web and are of particular interest due to their levels of bioactivity (Chauton et al., 2015). Regular consumption of EPA and DHA supplements has been shown to reduce inflammation, prevents cardiovascular disease (Adarme-Vega et al., 2012). DHA plays a vital role in maintaining the membrane fluidity of the brain and retina. Some of the DHA derived mediators are involved in reducing inflammation, and protection against injury (Saini and Keum, 2018). Recently the production of algal polyunsaturated fatty acids (PUFAs) has become more cost-effective compared to the production of biofuel and large number of producers has shifted their focus towards PUFA production (Sayeda et al., 2015).

Though microalgal products/co-products is recognized as nutritional supplements, only limited number of species was found successful in the market. Exploring robust algal candidate with fast growing ability and high production of preferable compound is imperative for the successful execution of the microalgal technique (Steinrücken et al., 2017). Hence, it is essential to acquire an adequate knowledge on the biochemical composition of each microalgae species in order to exploit their potential in different sectors (Matos et al., 2016). Further, extraction methods, and PUFA induction approaches like stress conditions need to be taken in account to identify high PUFA producer. Therefore, this review article initially emphasizes the significance of conventional and advanced lipid extraction methods and also the lipid accumulation in response to stress conditions. Further, the article focuses on the accumulation of PUFA in various algal species under different stress conditions and importance of PUFA.

2. Extraction of lipids

Efficient lipid extraction technique is imperative to obtain maximal lipid content from microalgae (Cuellar-Bermudez et al., 2015) and an effective cell disruption method with suitable solvent mixtures to recover maximal microalgal lipid were not established yet (Chauton et al., 2015). The extraction of lipids is usually done using a non-water miscible organic solvent (Adarme-Vega et al., 2012). Hexane is the most popular choice of organic solvent used for large- scale extractions. Fractional distillation is the best method used for the separation of unsaturated fatty acids from lipids whereas saturated lipids are precipitated by reducing the oil temperature (Adarme-Vega et al., 2012). The thick cell wall of several microalgae species blocks the release of intracellular lipids (Neto et al., 2013) and further, it is difficult to extract the lipids from the intracellular location of the cells without large amount of solvents. The basic and key requirement is to extract or separate oil without any interference of the contaminants (Araujo et al., 2013). The lipid extraction methods employed for microalgae are given in Table 1.

Current extraction methods used are fractional distillation, supercritical fluid extraction, ultra-sonication, soxhlet, solvent extraction, and microwave assisted extraction (Adam et al., 2012; Adarme-Vega et al., 2012; Araujo et al., 2013; Axelsson and Gentili, 2014; Biller et al., 2013; Neto et al., 2013; Ramluckan et al., 2014). However, extraction methods are of two types i.e., mechanical (oil expeller, microwave, sonication etc.,) and non-mechanical methods (Soxhlet extraction, supercritical fluid extraction etc.,) (Mathimani et al., 2017a, 2017c). In certain cases, mechanical and chemical methods were compared i.e., ultrasonication with ethanol (Wiyarno et al., 2011), microwave with hexane (Cravotto et al., 2008) and ultrasonic water bath with chloroform: methanol (Krienitz and Wirth, 2006). A basic extraction protocol includes a combination of organic solvents such as methanol and chloroform for the cell disruption and concurrent extraction of lipids from algal cells. In a solvent based extraction, soxhlet extraction with

Table 1
Extraction of lipids from microalgae.

Microalgae species	Extraction method	Product and key fatty acids observed	Lipid content (%)	References
Chlorella vulgaris, Scenedesmus dimorphus, Nannochloropsis sp.	Ultra-sonication and Enzyme extraction	Lipid	49.82, 46.81, 11.73	(Liang et al., 2012)
Chlorella vulgaris	Bligh and dyer, Chen, Folch, Hara and Radin methods assisted by Ultra-sound, and Soxhlet extraction	Lipid (palmitic acid, linoleic acid, oleic acid, linolenic acid)	52.5 ± 2.3 (without silica) and 41.4 ± 1.8 (with silica)	(Araujo et al., 2013)
Selenastrum minutum	Homogenization, Solvent extraction (Chloroform-Methanol 2:1 v/v)	Lipid (Palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, lignoceric acid)	1.26 ± 0.06–40.06 ± 0.25	(Axelsson and Gentili, 2014)
Chlorella sp.	Soxhlet extraction with three binary mixtures of Chloroform-Hexane 1:1), (Chloroform-Ethanol 1:1), Ethanol-Hexane 1:3)	Lipids	0.98, 11.76, 4.0.	(Ramluckan et al., 2014)
Nannochloropsis oculata	Ultrasound assisted extraction	Lipids (Myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, γ-linolenic acid, arachidonic acid, eicosapentaenoic acid)	0.21%	(Adam et al., 2012)
Chlorella minutissima, Thalassiosira fluviatilis, Thalassiosira pseudonana	Sonicated assisted solvent extraction	Lipids (NA)	15.5, 40.3, 39.5	(Neto et al., 2013)
Chlorogloeopsis fritschii Nannochloropsis oculata, Pseudochoricystis ellipsoidea	Hydrothermal microwave processing assisted solvent extraction	Lipids (NA)	0.5–37.5	(Biller et al., 2013)
Pavlova sp.	Supercritical CO_2 extraction with bead beating	Lipid	17.9	(Cheng et al., 2011)
Botryococcus braunii	Supercritical CO_2 extraction at 80 C	Lipid	14%	(Santana et al., 2012)

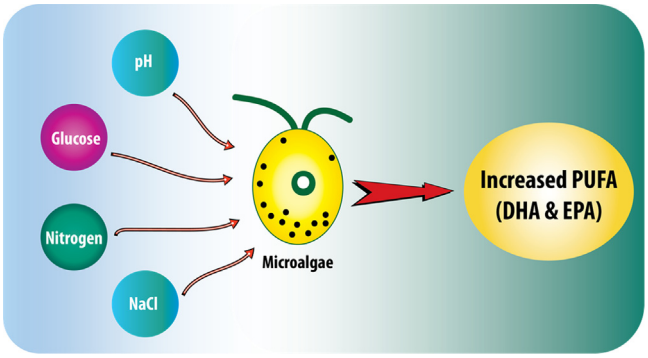

Fig. 1. Schematic illustration of the PUFA accumulation in response to various factors.

hexane (Halim et al., 2011), and chloroform/methanol binary solvent system in Bligh and Dyer method (Bligh and Dyer, 1959) is practiced to extract lipids from microalgae. In a recent study, direct solvent extraction containing chloroform and methanol has been found efficient in extracting maximal lipid at about 22% from marine microalga *Chlorella vulgaris* (Mathimani et al., 2017c). Extraction efficiency of different methods such as autoclaving, bead-beating, microwaves, sonication, and a 10% NaCl solution were compared. The lipid content of *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. were 5.4–11.9, 7.9–8.1, 10.0–28.6, 6.1–8.8, and 6.8–10.9 g/L with chloroform and methanol (1:1) using autoclaving, bead-beating, microwave method, sonication, and a 10% NaCl respectively and microwave facilitated approach was found as simple, and efficient microalgal lipid extraction technique (Lee et al., 2010). But in the recent past, several drawbacks were associated with these methods namely use of toxic solvents, and low product yield. To overcome these, SC-CO_2 has been used as an alternative extraction lipid extraction approach from microalgal species.

3. Accumulation of lipids in microalgae under stress conditions

Microalgae cultivated under stress generally accumulate lipids (Yen et al., 2013), and however, it leads to low lipid productivity due to the reduced growth rate (D'Alessandro and Antoniosi Filho, 2016). Concerning environmental factors, temperature and light intensity are the key factors affecting the lipid productivity of microalgae (Hindersin et al., 2014), whereas among the nutrients, sufficient or deficient supplementation of nitrogen and phosphorus influence the synthesis of biochemical components of algae (Rasdi and Qin, 2015). Several research reports showed higher lipid accumulation in microalgae grown under nitrogen and phosphorus limitation. Nitrogen limitation is a commonly used strategy to increase the lipids content in microalgal species. Under low nitrogen concentration, *Chlorella* sp. MACC-438, *Chlorella minutissima* MACC-452 and *Chlorella* sp. MACC- 728 were found to yield maximal lipid (Ördög et al., 2016). A study was carried out to assess the lipid contents of cyanobacteria and other classes of microalgae under normal and stress conditions for comparative analysis. It was observed that the lipid contents were increased by 10–20% in diatoms, green microalgae and other species under stress conditions which was comparatively higher than nitrogen sufficient conditions (Yen et al., 2013). Similarly, nitrogen stress increased the lipid content of *Nannochloropsis oculata* (Converti et al., 2009) and *C. pyrenoidosa* (Fan et al., 2014) at about 15% over control.

Increasing the lipid content of microalgae is also done by using biphasic strategy. The first method involves the cultivation of microalgal cells under sufficient nutrients conditions to stimulate higher growth and then the cells are collected and exposed to nutrient starvation for accumulation of the lipids (Mathimani et al., 2018b). Another study was undertaken to assess the total lipids productivity in *Haematococcus pluvialis* under nitrogen starvation and normal growth conditions. The lipids content of the *H. pluvalis* was found to be 34.85% and 15.61% under nitrogen starvation and normal condition respectively. The contents of the other identified fatty acids were found to be the same in both the given conditions (Damiani et al., 2010). In a recent study, the lipid content of marine microalga *Chlorella vulgaris* BDUG 91771 is altered by the cultivation conditions such as light intensity, and temperature and further, nitrogen and phosphorus concentration in medium changes the fatty acid profile of the strain intensely (Mathimani et al., 2018b).

4. Accumulation of PUFA in microalgae under the influence of abiotic factors

Fatty acids (FAs) are the most important components of marine microalgal sources as they are structurally diverse and have gained importance due to their taxonomic specificity (Mathimani et al., 2018b; Sahu et al., 2013). Further, fatty acid profile is conserved within the phylum but differ at inter-phylum level (Sahu et al., 2013). The fatty acids are being used as a biomarker to distinguish closely related microalgal species at generic levels. The profiling and analysis of fatty acids is growing as it leads to the identification of novel set of compounds with different functional groups (Lang et al., 2011). Among the fatty acids, polyunsaturated fatty acids (PUFA's) have more than one double bond in their long carbon chain structure and they are considered as high-value compounds in food and health industries. There are two classes of PUFAs ω-6 and ω -3, which are vital for human fitness and they are synthesized from linoleic acid and linolenic acid, respectively. In addition, alfa-linolenic acid, EPA, and DHA are the key ω-3 fatty acids while γ- linoleic acid and ARA are ω-6 fatty acids (Shanab et al., 2018). Microalgae are the richest source of PUFAs and notably, eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3) are the most important PUFA's due to their biological functions (Yen et al., 2013). In the marine food chain, DHA and EPA are synthesized by microalgae under various culture conditions including autotrophic, heterotrophic and mixotrophic conditions (Adarme-Vega et al., 2012). Further, various factors were also found to increase the PUFA content (Fig. 1). Three main strategies were suggested to improve EPA and DHA productivity i) regulating the cultivation conditions to maximize physiological potential for switching the cell metabolism to lipid and EPA + DHA synthesis; ii) escalate the biomass and lipid productivity through breeding of strains ii) Genetic engineering approach for EPA and DHA production (Chauton et al., 2015). Moreover DHA contents can be increased by various methods such as winterization, urea complexation, molecular distillation, $AgNO_3$-complexation etc. (Tang et al., 2011).

Several marine microalgae, and seaweeds belong to Phaeophyceae, Rhodophyceae, Dinophyceae, Chlorophyceae were explored for PUFAs (Shanab et al., 2018). EPA is predominant among various microalgal classes including *Bacillariophceae*, *Chlorophyceae*, *Chrysophyseae*, *Cryptophyceae*, *Eustigamatophyceae*, and *Prasinophyceae* whereas DHA is commonly found in dinoflagellate (*Crypthecodinium cohnii),* along with *Schizochytrium* and its related species. Variation in PUFA content was observed when algae were exposed to various stress conditions (Table 2). Studies have revealed a difference in the content of DHA and/or EPA in some of the species such as *Phaeodactylum tricornutum* (2.2–3.9% of DW) (Cerón-García et al., 2013), *Monodus subterraneus* (3% of DW) (Lu et al., 2002) and *Nannochloropsis* sp. (2.8–4.3%) (Camacho-Rodríguez et al., 2013). Many microalgal species including (*Chaetoceros*, *Isochrysis*, *Nannochloropsis*, *Tetraselmis*, *Thalassiosira*) produce sufficient amounts of DHA and EPA which are responsible for the development of various bivalve larvae. Under heterotrophic growing conditions, high amounts of DHA and EPA accumulation are observed in the strains of *Nannochloropsis*, *Phaeodactylum*, *Schizochytrium,* and *Thraustochytrium*. An EPA content of 39% of the total fatty acids was found in *Nannochloropsis* sp. and *Phaeodactylum tricornutum* whereas DHA content was found to be in the range of 30–40% of total fatty acids

Table 2
Accumulation of fatty acids in microalgae species under stress conditions.

Microalgae species	Growth conditions	Factors used/Stress	Response	References
Haematococcus pluvialis	Light density of 20 µmol m^{-2} s^{-1} with a diurnal cycle of 12/12 h light/dark at 22 ± 1 °C.	Nitrogen depletion, High and low temperature	Increased linoleic acid and PUFA contents.	(Lei et al., 2012)
Nannochloropsis gaditana	Aeration rate of 0.5 v v^{-1} min^{-1} under continuous illumination of 100 µE m^{-2} s^{-1} and at a temperature of 25 °C.	High concentration of nitrogen	EPA content was increased by 2.5 folds.	(Camacho-Rodriguez et al., 2013)
Haematococcus pluvialis	12/12 h light/dark cycle with 90 µmol photons m^{-2} s^{-1} light, 3.4 mM of sodium nitrate in medium, 0.30 cm/min CO_2 bubbling.	A. Continuous high light intensity B. Continuous high light intensity without nitrogen	Saturated fatty acids contents increased by 30.36% under condition A and 29.62% under condition B. PUFA contents were increased under condition B.	(Damiani et al., 2010)
Chlorella vulgaris, Scenedesmus, Spirulina platensis	Airlift photobioreactors at 25 ± 1 °C with a starting inoculum concentration of 0.05 g/L. NO_3 concentration 1500 mg L^{-1} (nitrogen sufficient) or 150 mg L^{-1} (nitrogen deficient.	Nitrogen depletion	Increased concentrations of Oleic acid (18:1) and Linoleic acid (18:2)	(Griffiths et al., 2012)
Pavlova lutheri	F/2 medium, grown at two different temperatures (15 ° and 25 °C)	Low temperature	PUFA (22:6) contents were increased from 10% to 14% and 33%.	(Tatsuzawa and Takizawa, 1995)
Phaeodactylum tricornutum	F/2 medium was used in which urea was increased 4-fold and phosphate to 2-fold and maintained at 130 µmol photons m^{-2} s^{-1} in 16:8 h light: dark cycle.	Low temperature	EPA and PUFA contents were increased	(Jiang and Gao, 2004)
Phaeodactylum tricornutum and Chaetoceros muelleri	Sterilized natural seawater enriched with f/2 medium containing nitrate (N-Nt), ammonium (N-Am) or urea (N-Ur) as nitrogen (N) source at final nitrogen concentration of 0.88 mM. Temperature: 18 ± 1 °C, Photon flux: 60 µmol quanta m^{-2} s^{-1}	High and low Light (UVR) under different nitrogen sources	*Phaeodactylum tricornutum*- Increased concentration of EPA and PUFA. *Chaetoceros muelleri*- Increased concentration of monounsaturated fatty acids.	(Liang et al., 2006)

for *Schizochytrium limacinum* and *Thraustochytrium* (Adarme-Vega et al., 2012). *Nannochloropsis* is considered as the most prominent strain in the commercial DHA and EPA production (Peltomaa et al., 2017). When microalgae species are grown in media with balanced carbon and nitrogen sources and under optimum pH with controlled temperature conditions, high DHA and EPA productivity is obtained. As an essential fatty acid, DHA could protect against neuro-generative diseases namely Alzheimer, Parkinson and multiple sclerosis as well (Shanab et al., 2018). Linoleic acid was found to be an effective formulation used in the treatment of skin hyperplasias (Santhosh et al., 2016).

Nutrients play a crucial role in producing high amounts of oil from *Schizochytrium* species. A study revealed 50% w/w of DHA content in *Schizochytrium* and high growth rate by controlling the concentration of glucose, nitrogen, and sodium. Further, external environmental factors like concentrations of oxygen, temperature, and pH were seen to influence the growth rate and productivity of DHA (Ward and Singh, 2005). Several studies have reported the influence of these factors on the growth and variation in the contents of DHA and EPA in microalgal species. The EPA content was increased from 20% to 30% in *Pavlova lutheri* when temperature was decreased to 15 °C (Tatsuzawa and Takizawa, 1995). Similarly, temperature decrease from 25–10 °C for 12 h, increased the EPA level in *Phaeodactylum tricornutum* (Jiang and Gao, 2004). The same species showed an increased content of EPA to 19.84% under UV light stress conditions (Liang et al., 2012). Induced mutagenesis is widely used to develop strains with required characteristics. A study on the UV-induced mutant strains of *Pavlova lutheri* showed an increase of more than 30% of the dry weight of DHA and EPA contents (Chauton et al., 2015). In another study, DHA content was increased up to 56.9% of total fatty acids in *Crythecodinium cohnii* ATCC 30556 when cultured in 9 g/L NaCl (Adarme-Vega et al., 2012). DHA production was further assessed by the effect of aeration in *Schizochytrium sp*. using fed-batch fermentation. In a stepwise manner, the aeration rate was set to 0.4 vol of air per volume of liquid (vvm)/min for the first 24 h, and then the air flow rate was changed to 0.6vvm for 96 h, and finally was shifted back to 0.4 vvm till the completion of the process. The changes in aeration rate caused increased cell density (71 g/L), higher levels of lipids (35.75 g/L), and increased DHA content (48.95%) (Yen et al., 2013).

Another study was carried out to evaluate the fatty acid content of *H. pluvialis* under the influence of various factors. The results revealed that low temperature (4 °C), increased iron concentrations (450 mM of $FeSO_4$), increased salinity (45 mM of sodium), high temperature (42 °C), and nitrogen depletion increased the contents of fatty acid by 1%, 9%, 22%, 23% and 32% respectively (D'Alessandro and Antoniosi Filho, 2016). *Nitzschia laevis* is found to be a promising source of EPA under heterotrophic culture conditions (glucose as a carbon and energy substrate), which yields 0.017 g g^{-1} EPA dry cell $weight^{-1}$ (Tan and Johns, 1996). Arachidonic acid (ARA) is a long chain (C20:4) PUFA, which are considered as a vital component of the bio-membranes (Shanab et al., 2018). Among the microalgal species, *Porphyridium purpureum*- a red alga is known to produce considerable amounts of ARA. ARA yield reached up to 40% of total fatty acids when the microalgae was cultured under stress conditions (increased salinity, optimum pH and temperature, and limited nutrients) (Shanab et al., 2018). *Parietochloris incisa* is a rich source of ARA as it possesses significant quantity of ARA (Bigogno et al., 2002). Metabolites of free ARA are essential for skeletal muscle, nervous system and immune system functioning for the resistance towards allergens and parasites and further, derivatives of oxidation-independent ARA are important for motivation processes, energy balance, emotion, pain and stress responses (Tallima and El Ridi, 2018).

5. Conclusion

Over the past few years, algal biotechnology industry has been growing rapidly and biotechnological potentials of microalgae is being

studied for various applications. Among the value added products from microalgae, successful extraction and purification of PUFAs from microalgal species is being done by various researchers and the role of PUFA in lowering the cholesterol levels and prevention of heart disease is also studied. In this article, extraction of lipid from microalgae grown under stress conditions using various techniques was discussed. Further, accumulation of PUFA in response to stress has also been presented. However, there is a lack of scientific knowledge about the significant biological properties of PUFA including nutraceuticals, pharmaceuticals etc. Recent efforts on extraction and purification of PUFAs from microalgae have been growing but more systematic studies are needed to identify economical methods to produce maximum PUFA from marine sources. Finally, it can be concluded that studies pertaining environmental factors needs to be addressed for obtaining high value products from microalgae and also to assess the sustainability of the processes.

Acknowledgement

Author Dr. T. Mathimani acknowledges Department of Science and Technology – Govt. of India for awarding INSPIRE Faculty (Grant no: DST/INSPIRE/04/2017/001922 & IFA17-LSPA87).

References

Adam, F., Abert-Vian, M., Peltier, G., Chemat, F., 2012. “Solvent-free” ultrasound-assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. Bioresour. Technol. 114, 457–465.

Adarme-Vega, T.C., Lim, D.K., Timmins, M., Vernen, F., Li, Y., Schenk, P.M., 2012. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb. Cell Factor. 11, 96.

Araujo, G.S., Matos, L.J., Fernandes, J.O., Cartaxo, S.J., Gonçalves, L.R., Fernandes, F.A., Farias, W.R., 2013. Extraction of lipids from microalgae by ultrasound application: prospection of the optimal extraction method. Ultrason. Sonochem. 20, 95–98.

Axelsson, M., Gentili, F., 2014. A single-step method for rapid extraction of total lipids from green microalgae. PloS One 9, e89643.

Bigogno, C., Khozin-Goldberg, I., Adlerstein, D., Cohen, Z., 2002. Biosynthesis of arachidonic acid in the oleaginous microalga Parietochloris incisa (Chlorophyceae): radiolabeling studies. Lipids 37, 209–216.

Biller, P., Friedman, C., Ross, A.B., 2013. Hydrothermal microwave processing of microalgae as a pre-treatment and extraction technique for bio-fuels and bio-products. Bioresour. Technol. 136, 188–195.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Camacho-Rodríguez, J., Cerón-García, M., González-López, C., Fernández-Sevilla, J., Contreras-Gómez, A., Molina-Grima, E., 2013. A low-cost culture medium for the production of Nannochloropsis gaditana biomass optimized for aquaculture. Bioresour. Technol. 144, 57–66.

Cerón-García, M., Fernández-Sevilla, J., Sánchez-Mirón, A., García-Camacho, F., Contreras-Gómez, A., Molina-Grima, E., 2013. Mixotrophic growth of Phaeodactylum tricornutum on fructose and glycerol in fed-batch and semi-continuous modes. Bioresour. Technol. 147, 569–576.

Chauton, M.S., Reitan, K.I., Norsker, N.H., Tveterås, R., Kleivdal, H.T., 2015. A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: research challenges and possibilities. Aquaculture 436, 95–103.

Cheng, C.-H., Du, T.-B., Pi, H.-C., Jang, S.-M., Lin, Y.-H., Lee, H.-T., 2011. Comparative study of lipid extraction from microalgae by organic solvent and supercritical CO_2. Bioresour. Technol. 102, 10151–10153.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P., Del Borghi, M., 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. Chem. Eng. Process.: Process Intensif. 48, 1146–1151.

Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M., Cintas, P., 2008. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. Ultrason. Sonochem. 15, 898–902.

Cuellar-Bermudez, S.P., Aguilar-Hernandez, I., Cardenas-Chavez, D.L., Ornelas-Soto, N., Romero-Ogawa, M.A., Parra-Saldivar, R., 2015. Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. Microb. Biotechnol. 8, 190–209.

D’Alessandro, E.B., Antoniosi Filho, N.R., 2016. Concepts and studies on lipid and pigments of microalgae: a review. Renew. Sustain. Energy Rev. 58, 832–841.

Damiani, M.C., Popovich, C.A., Constenla, D., Leonardi, P.I., 2010. Lipid analysis in Haematococcuspluvialis to assess its potential use as a biodiesel feedstock. Bioresour. Technol. 101, 3801–3807.

Fan, J., Cui, Y., Wan, M., Wang, W., Li, Y., 2014. Lipid accumulation and biosynthesis genes response of the oleaginous Chlorella pyrenoidosa under three nutrition stressors. Biotechnol. Biofuels 7, 17.

Griffiths, M.J., van Hille, R.P., Harrison, S.T., 2012. Lipid productivity, settling potential and fatty acid profile of 11 microalgal species grown under nitrogen replete and limited conditions. J. Appl. Phycol. 24, 989–1001.

Guihéneuf, F., Stengel, D.B., 2013. LC-PUFA-enriched oil production by microalgae: accumulation of lipid and triacylglycerols containing n-3 LC-PUFA is triggered by nitrogen limitation and inorganic carbon availability in the marine haptophyte Pavlova lutheri. Mar. Drugs 11, 4246–4266.

Halim, R., Gladman, B., Danquah, M.K., Webley, P.A., 2011. Oil extraction from microalgae for biodiesel production. Bioresour. Technol. 102, 178–185.

Hindersin, S., Leupold, M., Kerner, M., Hanelt, D., 2014. Key parameters for outdoor biomass production of Scenedesmus obliquus in solar tracked photobioreactors. J. Appl. Phycol. 26, 2315–2325.

Jiang, H., Gao, K., 2004. Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom Phaeodactylum tricornutum (bacillariophyceae) 1. J. Phycol. 40, 651–654.

Krienitz, L., Wirth, M., 2006. The high content of polyunsaturated fatty acids in Nannochloropsis limnetica (Eustigmatophyceae) and its implication for food web interactions, freshwater aquaculture and biotechnology. Limnol.-Ecol. Manag. Inland Waters 36, 204–210.

Kumar, K.S., Prasanthkumar, S., Ray, J., 2017. Biomass yield, oil productivity and fatty acid profile of Chlorella lobophora cultivated in diverse eutrophic wastewaters. Biocatal. Agric. Biotechnol. 11, 338–344.

Lang, I., Hodac, L., Friedl, T., Feussner, I., 2011. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biol. 11, 124.

Lee, J.-Y., Yoo, C., Jun, S.-Y., Ahn, C.-Y., Oh, H.-M., 2010. Comparison of several methods for effective lipid extraction from microalgae. Bioresour. Technol. 101, S75–S77.

Liang, K., Zhang, Q., Cong, W., 2012. Enzyme-assisted aqueous extraction of lipid from microalgae. J. Agric. Food Chem. 60, 11771–11776.

Liang, Y., Beardall, J., Heraud, P., 2006. Effects of nitrogen source and UV radiation on the growth, chlorophyll fluorescence and fatty acid composition of Phaeodactylum tricornutum and Chaetoceros muelleri (Bacillariophyceae). J. Photochem. Photobiol. B: Biol. 82, 161–172.

Lu, C., Fernández, F.A., Guerrero, E.C., Hall, D., Grima, E.M., 2002. Overall assessment of Monodus subterraneus cultivation and EPA production in outdoor helical and bubble column reactors. J. Appl. Phycol. 14, 331–342.

Mathimani, T., Baldinelli, A., Rajendran, K., Prabakar, D., Matheswaran, M., van Leeuwen, R.P., Pugazhendhi, A., 2018a. Review on cultivation and thermochemical conversion of microalgae to fuels and chemicals: process evaluation and knowledge gaps. J. Clean. Prod.

Mathimani, T., Bhumathi, D., Ahamed, T.S., Dineshbabu, G., Deviram, G., Uma, L., Prabaharan, D., 2017a. Semicontinuous outdoor cultivation and efficient harvesting of marine Chlorella vulgaris BDUG 91771 with minimum solid co-precipitation and high floc recovery for biodiesel. Energy Convers. Manag. 149, 13–25.

Mathimani, T., Kumar, T.S., Chandrasekar, M., Uma, L., Prabaharan, D., 2017b. Assessment of fuel properties, engine performance and emission characteristics of outdoor grown marine Chlorella vulgaris BDUG 91771 biodiesel. Renew. Energy 105, 637–646.

Mathimani, T., Mallick, N., 2018. A comprehensive review on harvesting of microalgae for biodiesel–Key challenges and future directions. Renew. Sustain. Energy Rev. 91, 1103–1120.

Mathimani, T., Nair, B.B., 2016. Evaluation of microalga for biodiesel using lipid and fatty acid as a marker–a central composite design approach. J. Energy Inst. 89, 436–446.

Mathimani, T., Pugazhendhi, A., 2018. Utilization of algae for biofuel, bio-products and bio-remediation. Biocatal. Agric. Biotechnol.

Mathimani, T., Uma, L., Prabaharan, D., 2015. Homogeneous acid catalysed transesterification of marine microalga Chlorella sp. BDUG 91771 lipid–an efficient biodiesel yield and its characterization. Renew. Energy 81, 523–533.

Mathimani, T., Uma, L., Prabaharan, D., 2017c. Optimization of direct solvent lipid extraction kinetics on marine trebouxiophycean alga by central composite design–bioenergy perspective. Energy Convers. Manag. 142, 334–346.

Mathimani, T., Uma, L., Prabaharan, D., 2018b. Formulation of low-cost seawater medium for high cell density and high lipid content of Chlorella vulgaris BDUG 91771 using central composite design in biodiesel perspective. J. Clean. Prod. 198, 575–586.

Matos, Â.P., Feller, R., Moecke, E.H.S., de Oliveira, J.V., Junior, A.F., Derner, R.B., Sant'Anna, E.S., 2016. Chemical characterization of six microalgae with potential utility for food application. J. Am. Oil Chem.' Soc. 93, 963–972.

Moreno-Garrido, I., 2008. Microalgae immobilization: current techniques and uses. Bioresour. Technol. 99, 3949–3964.

Morowvat, M.H., Ghasemi, Y., 2016. Evaluation of antioxidant properties of some naturally isolated microalgae: Identification and characterization of the most efficient strain. Biocatal. Agric. Biotechnol. 8, 263–269.

Neto, A.M.P., de Souza, R.A.S., Leon-Nino, A.D., da Costa, J.Da.A., Tiburcio, R.S., Nunes, T.A., de Mello, T.C.S., Kanemoto, F.T., Saldanha-Corrêa, F.M.P., Gianesella, S.M.F., 2013. Improvement in microalgae lipid extraction using a sonication-assisted method. Renew. Energy 55, 525–531.

Ördög, V., Stirk, W.A., Bálint, P., Aremu, A.O., Okem, A., Lovász, C., Molnár, Z., van Staden, J., 2016. Effect of temperature and nitrogen concentration on lipid productivity and fatty acid composition in three Chlorella strains. Algal Res. 16, 141–149.

Peltomaa, E., Johnson, M.D., Taipale, S.J., 2017. Marine cryptophytes are great sources of EPA and DHA. Mar. Drugs 16, 3.

Ramluckan, K., Moodley, K.G., Bux, F., 2014. An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet

extraction method. Fuel 116, 103–108.
Rasdi, N.W., Qin, J.G., 2015. Effect of N:P ratio on growth and chemical composition of Nannochloropsis oculata and Tisochrysis lutea. J. Appl. Phycol. 27, 2221–2230.
Sahu, A., Pancha, I., Jain, D., Paliwal, C., Ghosh, T., Patidar, S., Bhattacharya, S., Mishra, S., 2013. Fatty acids as biomarkers of microalgae. Phytochemistry 89, 53–58.
Saini, R.K., Keum, Y.-S., 2018. Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance—a review. Life Sci.
Santana, A., Jesus, S., Larrayoz, M., 2012. Supercritical carbon dioxide extraction of algal lipids for the biodiesel production. Procedia Eng. 42, 1755–1761.
Santhosh, S., Dhandapani, R., Hemalatha, R., 2016. Bioactive compounds from micro-algae and its different applications—a review. Adv. Appl. Sci. Res 7, 153–158.
Saravanan, A.P., Mathimani, T., Deviram, G., Rajendran, K., Pugazhendhi, A., 2018. Biofuel policy in India: a review of policy barriers in sustainable marketing of biofuel. J. Clean. Prod. 193, 734–747.
Sayeda, M., Ali, G.H., El-Baz, F.K., 2015. Potential production of omega fatty acids from microalgae. Int. J. Pharm. Sci. Rev. Res 210–215.
Shanab, S.M., Hafez, R.M., Fouad, A.S., 2018. A review on algae and plants as potential source of arachidonic acid. J. Adv. Res.
Steinrücken, P., Erga, S.R., Mjøs, S.A., Kleivdal, H., Prestegard, S.K., 2017. Bioprospecting North Atlantic microalgae with fast growth and high polyunsaturated fatty acid (PUFA) content for microalgae-based technologies. Algal Res. 26, 392–401.
Tallima, H., El Ridi, R., 2018. Arachidonic acid: physiological roles and potential health benefits–A review. J. Adv. Res. 11, 33–41.
Tan, C.K., Johns, M.R., 1996. Screening of diatoms for heterotrophic eicosapentaenoic acid production. J. Appl. Phycol. 8, 59–64.
Tang, S., Qin, C., Wang, H., Li, S., Tian, S., 2011. Study on supercritical extraction of lipids and enrichment of DHA from oil-rich microalgae. J. Supercrit. Fluids 57, 44–49.
Tatsuzawa, H., Takizawa, E., 1995. Changes in lipid and fatty acid composition of Pavlova lutheri. Phytochemistry 40, 397–400.
Ward, O.P., Singh, A., 2005. Omega-3/6 fatty acids: alternative sources of production. Process Biochem. 40, 3627–3652.
Wiyarno, B., Yunus, R., Mel, M., 2011. Extraction of algae oil from Nannocloropsis sp.: a study of soxhlet and ultrasonic-assisted extractions. J. Appl. Sci. 11, 3607–3612.
Yen, H.-W., Hu, I.-C., Chen, C.-Y., Ho, S.-H., Lee, D.-J., Chang, J.-S., 2013. Microalgae-based biorefinery–from biofuels to natural products. Bioresour. Technol. 135, 166–174.