



Review Article

Advances in microalgae cultivation and extraction for sustainable omega-3 oil production: A review on EPA and DHA sourcing

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ABSTRACT

These are nutrients that must be derived from dietary sources since the human body lacks some enzymes that are necessary for the synthesis of long-chain fatty acids with more than 18 carbons. These can be acquired from microalgae—a rich source reservoir of bioactive compounds, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play very important roles in maintaining human health and preventing chronic diseases. The paper reviews different methods or strategies to ensure the stability or optimization of microalgae cultures for the extraction of high-value, long-chain lipids. According to various studies, microalgae have emerged as one promising source for the production of omega-3 fatty acids in a very sustainable and efficient manner, hence prospectively assuring positive yields at both the research and industrial levels. The current review describes the recent advances made in the production of PUFAs from microalgae, including their extraction, based on economic and scalable cultivation techniques. Further, the effect of environmental variables, such as temperature, light, salinity, and nutrients, on the production of lipids from microalgae, is discussed. In this review, state-of-the-art upstream and downstream processes regarding oleaginous microalgae are presented, jointly with innovative extraction methods able to overcome their environmental concerns. This review therefore provides critical analysis necessary to bridge the gap between research and industrial application and also gives insight into how microalgae can be sustainably exploited for nutritional and pharmaceutical purposes.

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1. Introduction

The long chain ω -3 fatty acids (ω -3 PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) display an essential biological role. The molecular weight of the DHA structure is $328.5 \text{ g. mol}^{-1}$, and the molecular weight of EPA is $302.5 \text{ g. mol}^{-1}$.

The prominent benefits of EPA and DHA are stated by the European Food Safety Authority (EFSA), due to the claims we know that these fatty acids improve some diseases like cancer prevention, psychological problems, nervous system, and coronary heart disease (Koller *et al.*, 2014). DHA is one of the components of human breast milk and is important for an inexperienced person's nervous system and brain (Nettleton, 1993). The knowledge development regarding the positive impact of DHA and EPA on human metabolisms such as reduced hypercholesterolemia, hypertriglyceridemia, and high-density lipoprotein (Hamed *et al.*, 2015).

Recently, studies have shown utilization ω -3 in the diet plays a neuroprotector role and prevents some diseases such as Alzheimer's, depression, and Parkinson's (Eriksdotter *et al.*, 2015; Mocking *et al.*, 2016). Research suggests that different blood levels of EPA and DHA between populations of diverse regions, with diverse seafood intakes. For example, the level of EPA and DHA in the populated Sea of Japan is the highest (Stark *et al.*, 2016).

Medical proven synthesizes long-chain fatty acids more than 18 carbons in the human body lack enzymes, so should be obtained these from food (Hamed *et al.*, 2015). The most unstable oxidation and unsaturated fatty acids are EPA and DHA (Beigi Keleshteri *et al.*, 2024).

Microorganisms that contain more than 20% lipid in their cellular compartments are named oleaginous microorganisms, and they can synthesize PUFAs from Hydrocarbonated molecules (Patel *et al.*, 2020). Microalgae have promised specifications, like being unicellular, inhabiting marine water and freshwater environments, and being photoautotrophic and heterogenous organisms (Olaizola, 2003). Microalgae can be eukaryotic or prokaryotic and, in turn, they can be a valuable source of some

products such as cosmetic, biofuel, pharmaceutical, medical, and food industries (Olaizola, 2003). Microalgae have the preventive potential of water pollution and greenhouse problems because they can fix carbon dioxide with photosynthesis, fix nitrogen and phosphorous, and absorb heavy metals (Harun *et al.*, 2010). Marine microalgae possess multitudes quantities of PUFAs due to their survival in brine water, but freshwater microalgae have a lower content of PUFAs (Dewapriya & Kim, 2014).

For the extraction of lipids from microalgae, some properties should be scrutiny. Cultivation conditions and strain origin are impactful on composition and quantity. Microalgae react and transform in intracellular environments with an environmental alteration. Therefore, manipulated photoperiod, temperature, pH, and so on... cause efficiency with beneficent commercial value (Pal *et al.*, 2011).

In recent years, food processing and extraction techniques have evolved significantly, incorporating advanced technologies to enhance efficiency and product quality. Among these innovations, ultrasound-assisted methods combined with machine learning have demonstrated considerable potential in improving the quality assessment of food products (Farshbaf Aghajani *et al.*, 2023; Chamgordani *et al.*, 2024; Farshbaf Aghajani *et al.*, 2024). Extraction methods adjust to increase production efficiency. Some development techniques for extraction lipids used in the research reported are explained here, for example, Soxhlet, Folch, Supercritical fluid, pressurized liquid (PLE), osmotic shock, ultrasonic-assisted, Supercritical fluid extraction (SFE).

According to Action, (2020), the total aquaculture production and fisheries in the whole world reached 178.5 million tonnes, about 87 percent of that, spent on human consumption. Sources of oil extraction are crops, oily fishes, some species of bacteria, marine algae, fungi, oysters, and shrimps are rich sources of long-chain fatty acids (Abedi & Sahari, 2014). Briefly, fish do not qualify for synthesizing DHA and EPA, whereas consume them from zooplankton as a

Table1.

The amount of content of lipid in oily microalgae.

Microalgae	Oil yield (%)	Type of omega-3	Reference
<i>Pavlova lutheri</i>	41.5	EPA + DHA	(Chaturvedi & Fujita, 2006)
<i>Nannochloropsis</i> sp.	38 – 39	EPA	(Chaturvedi & Fujita, 2006)
<i>Nannochloropsis oceanica</i>	23.4	EPA	(Patil <i>et al.</i> , 2007)
<i>Nannochloropsis salina</i>	~28	EPA	(Van Wageningen <i>et al.</i> , 2012)
<i>Nannochloropsis salina</i>	44.2 ± 2.30	EPA	(Safafar <i>et al.</i> , 2016)
<i>Pinguicoccus pyrenoidosusa</i>	22.03	EPA + DHA	(Safafar <i>et al.</i> , 2016)
<i>Thraustochytrium</i> sp.	45.1	EPA + DHA	(Scott <i>et al.</i> , 2011)
<i>Chlorella minutissima</i>	39.9	EPA	(Yongmanitchai & Ward, 1991)
<i>Phaeodactylum tricornutum</i>	25.8	EPA	(Reis <i>et al.</i> , 1996)
<i>Aurantiochytrium</i> sp.	40	DHA	(Hong <i>et al.</i> , 2011)
<i>Cryptocodinium cohnii</i>	31.1	DHA	(De Swaaf <i>et al.</i> , 2003)
<i>Schizochytrium</i> sp.	32.5	DHA	(Wu <i>et al.</i> , 2005)
<i>Isocrysis galbana</i>	~28.0	EPA + DHA	(Fang <i>et al.</i> , 2020)
<i>Pavlova viridis</i>	36.0	EPA + DHA	(Hu <i>et al.</i> , 2008)
<i>Dunaliella salina</i>	21.4	EPA	(Bhosale <i>et al.</i> , 2010)
<i>Phaeodactylum tricornutum</i>	31.4 ± 3.3	EPA	(Steinrücken <i>et al.</i> , 2018)
<i>Isochrysis galbana</i>	3.3 ± 0.5	EPA	(Señoráns <i>et al.</i> , 2020)
<i>Teleaulax amphioxeia</i>	17.9	EPA	(Lee <i>et al.</i> , 2019)
<i>Gymnodinium smaydae</i>	8.41 ± 0.32	EPA	(Lim <i>et al.</i> , 2020)

part of their diet. By contrast, zooplankton consume EPA and DHA from feeding on microalgae (Ackman *et al.*, 1964). Therefore, the main sources of DHA and EPA are naturally marine (Tocher *et al.*, 2019). Iran is a vast country that includes 1,636 million square kilometers of land and Merely 14% of the land of Iran is cultivable for agriculture. Hence, accept that receiving long-chain fatty acids from marine water sources to a few other needed products can be provided from the agricultural land. The coastline of Iran is 2,440 km long and an amount of 30% of the Caspian Sea (FAO, 2023). The particular proper of microalgae is capable of a high growth rate in the condition of heterotrophic, autotrophic, and mixotrophic.

Processing microalgae for production oil contains some steps. Depending on the strain of microalgae, techniques could be different (Patel *et al.*, 2020):

1. Cultivation of microalgae
2. Separation of biomass from the cultivation medium
3. Oil extraction from obtained algae
4. Purification of the oil.

2. Discussion of the sources of oils with microalgae oil

2.1. Fish

Some fish have chemical pollution (Pulz & Gross, 2004), heavy metals that are toxic such as mercury and copper, and organic pollutants such as polychlorinated biphenyls (PCBs) and dioxins (Domingo, 2007). However, natural sources of EPA and DHA are extracted from fish and shellfish, and it is the primary source of PUFAs for humans and animals obtained from fish (Ward & Singh, 2005; Hosseini *et al.*, 2024). Algae oil is the best alternative to fish oil, which eliminates taste and odor problems (Valencia *et*

al., 2007). In consequence, microalgae products are known to be safe for human or animal consumption.

2.2. Crop

The use of microalgae is advantageous and incomparable with crops. Microalgae have a shorter rate of maturation, higher growth rate, and use less land area than crops (Lee *et al.*, 2010). Crops require genetic modification for the production of PUFAs (Ursin, 2003). Algae only need sunlight, carbon dioxide, nitrogen, and phosphate. Algae can grow in freshwater or brine water (Rodolfi *et al.*, 2009).

2.3. Krill

Another valuable source of EPA and DHA is krill, which are inaccessible to the population of humans, known as whale food, and live in the poles (Burri & Johnsen, 2015). These species are small and have the largest population of animals, crustaceans, and live in arctic seas. Krill oil contains the perfect part of DHA and EPA bounded phospholipids with phosphatidylcholine efficiency of krill more than fish oil (Subbaiah *et al.*, 2016).

2.4. Diatoms

Diatoms are photobioreactors (PBRs) and unicellular and photosynthetic brown microalgae. Some diatoms like *phaeodactylum tricornutum* contain a high amount of PUFAs like carotenoids (Gille *et al.*, 2016; Zhang *et al.*, 2018). Overexpression of the malic enzyme in transgenic cells in diatoms can increase the content of total lipids significantly by 2.5 fold of dry weight of cells (Xue *et al.*, 2015; Masoumi Varaki *et al.*, 2024).

2.5. Microalgae

Microalgae are unicellular and have extreme adaptation ability to diverse environmental situations, including cyanobacteria protists, and are also attainable in freshwater and brine water. The richest sources of EPA are marine microalgae compared with other microorganisms (Geada *et al.*, 2021).

In addition to marine microalgae, eustigmatophytes contain a high amount of EPA

and one of the significant sources of eustigmatophytes is *Nannochloopsis* sp. have been plausible studies on EPA potential (Chauton *et al.*, 2015). Depending on the species, microalgae biomass had a species valuable compound. Some of the oily algae are explained in Table 1.

2.5.1. *Chlorella vulgaris*

It has high-value protein content and the capability to merge triacylglycerides (TAG) with monounsaturated fatty acids (MUFAs) (Ursu *et al.*, 2014).

2.5.2. *Nannochloropsis gaditana*

It is oleaginous, For the first time, isolated from Cadiz Bay, in Spain (Figueroa *et al.*, 1997). It is a valuable source of lipids, with a range of lipid content between 25 to 45 percent in biomass dry (Chua & Schenk, 2017), whereas the content of polar lipids (phospholipids and glycolipids) and triglycerides. The structure of cell membranes, which have high compounds of EPA is polar lipids (Schüler *et al.*, 2017). Therefore, a new extraction method based on green chemistry and using organic solvent can be applied (Kumar *et al.*, 2017; Noormohammadi *et al.*, 2023). This microalgae bilayer wall and the external layer are hydrophobic. Therefore, it had a protective structure (Scholz *et al.*, 2014).

The amount of EPA yield regarding *Nannochloropsis* is explained in Table 3. *Nannochloropsis* and *Phaeodactylum* contain a high amount of EPA, and *Schizochytrium* sp. and *C. cohnii* are significant sources of DHA (Adarme-Vega *et al.*, 2012). *Thraustochytrium* and *Schizochytrium* are heterotrophic microalgae, and *C. cohnii* is a dinoflagellate. These three species possess the rich value of EPA and DHA and the lower value of other PUFAs, and they have practicable usage for humans (Fedorova-Dahms *et al.*, 2011; Martins *et al.*, 2013).

2.5.3. *Isochrysis*

The most particular property has no cell wall, omega-3 PUFA efficiency, and high lipid for extraction. It is a photosynthetic yield, oleaginous microalgae, and easily cultivated in the industrial bioreactor (Che *et al.*, 2019).

2.5.4. *Phaeodactylum tricorutum*

It is a diatom and the only species that belongs to the family *Phaeodactylum* and belongs to *Bacillariophyceae*. The former classification was identified as a *Nitzschia closterium* (Lewin *et al.*, 1958). Generally, these microalgae are found in brine and brackish water. *P. tricorutum* has an endurance of high pH and non-susceptibility to low light density, even having the authority to grow in low light intensity (Spilling *et al.*, 2013). *P. tricorutum* microalga is a rich source of EPA, by reportage that yields 25.8 % EPA (Reis *et al.*, 1996) and about 31.4 % EPA (Steinrücken *et al.*, 2018).

2.5.5. *Cryptocodinium cohnii*

Microalgae found in marine water that exist in heterotrophic conditions is known as a significant source of DHA and yields a vast amount of DHA (about 30 – 50 %) in Harrington and Holz Jr, (1968). *C. cohnii* provides a carbon source from glucose to produce DHA (Kyle, 1996). *C. cohnii* is a dinoflagellate that provides a carbon source from glucose to produce DHA content (Kyle, 1996; Martins *et al.*, 2013). Habitat *C. cohnii* microalgae are in the littoral, neritic area, and brackish water (Beam & Himes, 1987).

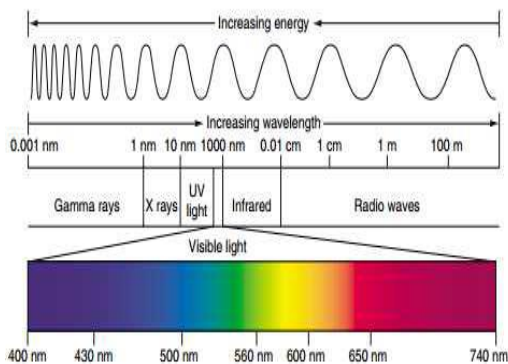


Figure 1. Visible light and electromagnetic spectrum (Schulze *et al.*, 2014).

3. Cultivation of microalgae

Microalgae can grow and survive in heterotrophic, photoautotrophic, photoheterotrophic, and mixotrophic conditions (Daneshvar *et al.*, 2021). Photoautotrophic

microalgae receive energy from light and receive carbon from inorganic carbon as a source of carbon for photosynthesis to produce organic matter (Verma & Srivastava, 2018). Heterotrophic microalgae have a higher rate of growth with photosynthesis, higher density of cells, and highly productive biomass, as long as they are in dark conditions without light and utilize organic carbon (Chen, 1996; Perez-Garcia *et al.*, 2011; Morales-Sánchez *et al.*, 2015).

In Heterotrophic cultivation, another Heterotrophic microorganism can be predominant, so the cultivation environment needs sterilizing (Morales-Sánchez *et al.*, 2015; Daneshvar *et al.*, 2021). Mixotrophic microalgae are included in both heterotrophic and photoautotrophic specifications, absorbed simultaneously by the externality of organic carbon and CO_2 (Cheirsilp & Torpee, 2012). The main reason for preventing exposure of microalgae into the market is the arduous cultivation system (Geada *et al.*, 2021).

Mitochondria degradation the stored organic carbon in the cytoplasm to create ATP by cell (lipid or carbohydrates) (Mooij *et al.*, 2013). The function of photosynthesis is shown in Figure 2. Supply nutrients, light intensity, and carbon dioxide are the main parameters in a biomass environment. During the use of artificial light, it is significant to prevent radiation of white light that is owing to green light not absorption by algae (~ 500 to 600 nm shown in Figure 1) (Schulze *et al.*, 2014).

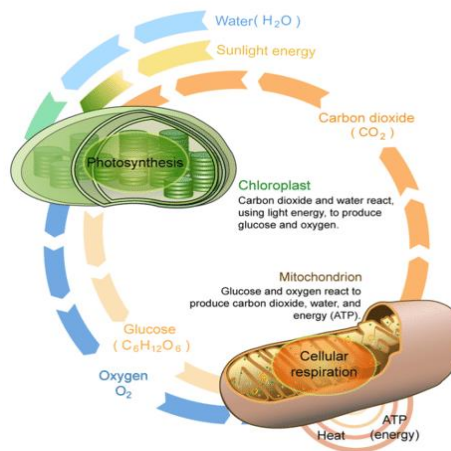


Figure 2. The function of photosynthesis.

According to the studies, the omega-3 content of *Nannochloopsis oculata* can be obtained by mutagenesis with UV radiation (Moha-León *et al.*, 2019). In the *N.oceanica* microalgae, EPA content can increase with co-cultivation with probiotic bacteria causing the content of EPA to increase 2.5-fold (Liu *et al.*, 2020).

The cultivation of microalgae is strongly dependent on the light intensity, and light distribution in the biomass environment is non-uniformed. Therefore, most of the light is absorbed by the surface cell, so the radiated distribution is shown in Figure 3 (Takache *et al.*, 2012).

During the duration of the culture observed, the concentration of cells in a bright zone (G_c) course to appear in a dark zone (G_z) with lower irradiance. This incident caused a reduction in the productivity of biomass. So that has high productivity, periodic harvest is exigency and requires optimization of the light regime.

Controlling pH, temperature, and light supplication optimally is the most significant issue in microalgae cultivation. Cultivation conditions should be the same as natural environmental conditions, as shown in Figure 4 and 5. (Varshney *et al.*, 2015).

3.1. Cultivation system

Briefly, two methods in phototrophic microalgae culture to produce the intended product: one is an open system, and the other is a closed system (photobioreactors or PBR). (Pulz, 2001; Greenwell *et al.*, 2010; Grobbelaar, 2010). Manufacture in open systems is frequently cheap and includes vast tank content, but after that has low efficiency and low production. Present close systems increase contraction costs, by contrast, possess more high efficiency and decrease harvest costs.

3.1.1. Open system

Temperature, and CO_2 ; supplied by the natural environment, however, it has serious problems like high evaporation of water and low volumetric product (Marsullo *et al.*, 2015).

3.1.2. Close system

Control pH, CO_2 , temperature, content of nutrients, light intensity, and minimization

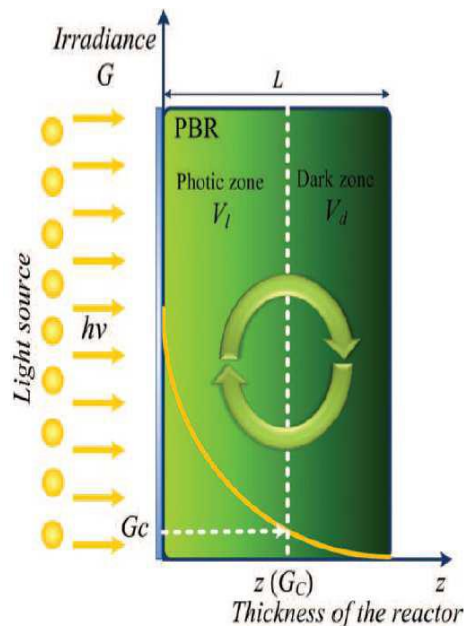


Figure 3. Radiated distribution (Takache *et al.*, 2012).

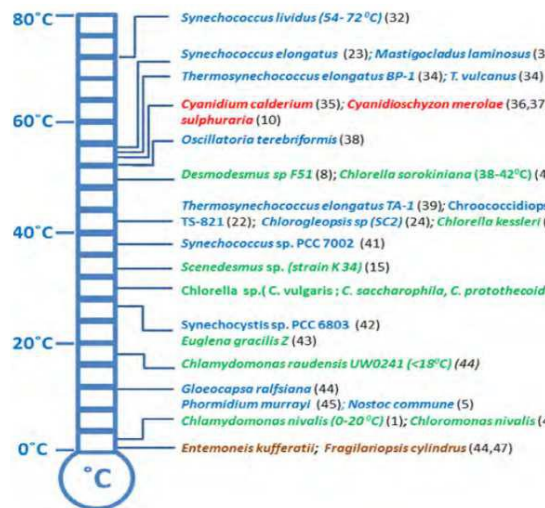


Figure 4. The optimization temperature for some microalgae, algae, cyanobacteria, and diatoms (Varshney *et al.*, 2015).

contamination are properties of the closed system.

4. How microalgae can be beneficial in oil

compounds

Algae metabolism can be changed by some parameters such as salinity, water temperature, nutrients, and light and it has to adapt quickly to continuously survive as a result of this incident algae produce secondary metabolites (Plaza *et al.*, 2008).

In the condition of nitrogen limitation, reduce the synthesis of nitrogen compounds such as protein, nucleic acid, and chlorophyll. In addition, algae continue to grow and carbon metabolic processes change to manufacture lipid or carbon hydrate instead of protein (Li *et al.*, 2008). If a toxic solvent is applied in the extraction method, the end products should be protected by the regulations in the industry (Bordoloi & Goosen, 2020).

The main environmental parameters, such as temperature, percentage of dissolved oxygen, light intensity, and aeration rate, play an essential role in the growth rate and content of PUFAs and long-chain lipids (Daneshvar *et al.*, 2021). Also, mutagenesis in *Nannochloopsis* sp. can be achieved by chemical compounds such as nitrosoguanidine and N-Methyl-N-nitrosourea (Wang *et al.*, 2016).

In some microalgae, mutagenesis causes a reduction in the rate of growth but increases PUFAs, including *Nannochloopsis salina* with ethyl methyl sulfonate (EMS) mutagenesis (Beacham *et al.*, 2015).

Multiple techniques are utilized to produce lipids in microalgae biomass: 1) At first, microalgae are cultivated in a nutrient cultivation environment to reach their production. Then, the nutrient-free cultivation environment for stopped products in biomass to increase the quantities of lipid content. The studies conducted in this regard include (Takeshita *et al.*, 2014).

This technique operates in large-scale execution of cultivations and commercial scales. At first, microalgae biomass grows under the condition of attenuating nutrients and nitrogen in the cultivation environment, and microalgae completely consume nutrients. Then biomass commences to stash lipid content or starch content (Griffiths *et al.*, 2014; Zhao *et al.*, 2014).

4.1. Nitrogen limitation

Reducing Nitrogen is a lipid increase strategy, as gaining more TGA products while getting the stress of reducing nitrogen. The synthesis of EPA from Nitrogen Limited should be discussed to understand the process. During the stress by nitrogen alteration of lipids due to remodeling of lipids (Han *et al.*, 2017). Studies show that, in *Nannochloopsis* sp. during the limited of nitrogen, EPA content decreases (Hulatt *et al.*, 2017). Another study observed that nitrogen depletion reduces the amount of EPA content and augments Palmitoleic acid in a particular strain of microorganisms called eustigmatophytes (Wang *et al.*, 2018).

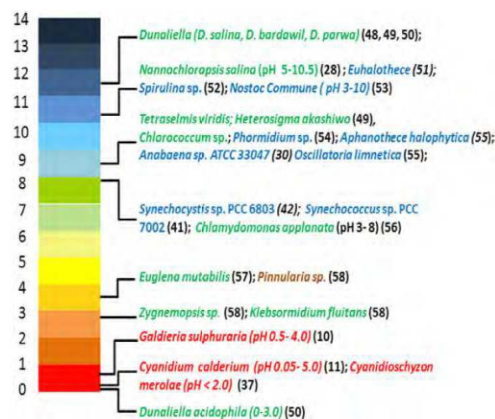


Figure 5.

Some limitations of pH for microalgae and cyanobacteria (Varshney *et al.*, 2015).

4.2. Light intensity

Under nitrogen depletion, in light conditions, TGA content was investigated and found that it had high efficiency because of photosynthesis (Mulders *et al.*, 2014). In the *C. vulgaris* efficiency of light intensity and photoperiod was investigated and observed that in photoperiods of 16:8 h, and 62.5 $\mu\text{mol. m}^{-2} \text{ s}^{-1}$ light intensity caused maximum biomass (Amini Khoeyi *et al.*, 2012). In the greenhouse environment, obtain the passable efficiency of photosynthesis using LED (light emitting diodes) (Scholz *et al.*, 2014). The studies of the efficiency of a diversity of wavelength on EPA and growth rate found that a directly used combination of blue light and red light can improve the efficiency of EPA and

deficiency electricity utilization (Chen *et al.*, 2015).

4.3. Temperature

During studies on temperature on eight species of microalgae and their lipid compounds, researchers found that the efficiency of EPA content at lower temperatures is more optimal than at higher temperatures in *Nannochloropsis* sp. Microalgae (Aussant *et al.*, 2018). Cepák *et al.* (2014) studied the effect of all conditions of cultivation on EPA content and PUFA compounds in *Trachydiscus minutus* microalgae and found the quantity of EPA at 20°C is more than the quantity of EPA at 28°C. Another study on *Prophyridium croventum* microalgae observed that EPA content is more than ARA (ARA/EPA). In other words, EPA content increased, and consequently, ARA content decreased, and this occurrence was investigated at 20 °C (Asgharpour *et al.*, 2015). Convention the temperature to 20°C, instead of 25°C, caused an increase in the EPA content of 45% from 20% quantity of total fatty acid (Seto *et al.*, 1984). In consequence, stress caused by reduced temperature might increase EPA content in microalgae.

4.4. Salinity stress

In *P. tricornutum* microalgae, increased EPA content with a hypersalinity environment, then microalgae biomass subjected to temperature, the intensity of light, and Fulvic acid cultivation (Wang *et al.*, 2019).

5. Harvesting

The main challenging factor of harvesting is controlling the process to limit increased costs (Vandamme *et al.*, 2013). Effective factors in the harvest technique include the shape and size of microalgae, resilience of organisms, and quality and quantity of revenue. Moreover, culture suspension density is critical in the following process (Muylaert *et al.*, 2017). Separation of water from the medium of culture referred to as drying or dewatering microalgae cultivated in an open system has a remarkable cost mainly because they are relatively dilute (Bosma *et al.*, 2010). Briefly, traditional mechanical methods

for harvesting are filtration, flotation, centrifugation, and sedimentation (Vasistha *et al.*, 2021). The chemical method for harvesting is flocculation (Christenson & Sims, 2011).

5.1. Centrifugation

The most prevalent method and high-value production in the industry is centrifugation (Grima *et al.*, 2003). Centrifugation is more productive in comparison with the pH-induced flocculation method in *N. oculata* microalgae (Borges *et al.*, 2016). *P. tricornutum* microalgae are another rich source of EPA and utilized centrifugation had more efficiency of EPA content in comparison with the Alkaline flocculation method (Vandamme *et al.*, 2018). As a consequence of all these, centrifugation is an extraordinary method, but it is costly because of the application of a bunch of energy to concentrate the biomass of microalgae.

5.2. Filtration

Backwashing membranes are used in part of the filtration method, and utilized before centrifugation as pre-concentrated for *N. oculata* microalgae harvesting (De Baerdemaeker *et al.*, 2013). Put to use the filtration before centrifugation, increase efficiency and reduce energy consumption in the harvest of *Nannochloropsis* sp. Biomass (Khairuddin *et al.*, 2019).

5.3. Flocculation

In the flocculation method, some flocculants, and coagulated cells, and used for primitive dewatering before centrifugation. Flocculation had acceptable results during application for freshwater microalgae, but when used for marine microalgae, results could not be receivable, because of ions content (Vandamme *et al.*, 2013). Put-to-use cationic polymers Cn solved this problem, such as Tanfloc used for flocculation *N. oculata* and reports indicate 99% efficiency (Roselet *et al.*, 2016). Food-grade foresaid flocculation is chitosan and its investigation on *Nannochloropsis* sp. with 99% efficiency (Chua *et al.*, 2019). Chu *et al.* (2020) used chitosan that exploitation from a mushroom in a cultivation environment with 2000liter content at 25 ppm

for *N. oceanica* microalgae. These compounds are costly for use in industry. Therefore, the utilization of affordable sources, such as magnetic nanoparticles that are recyclable compounds, could be an alternative. Flocculation with induction pH that used NaOH showed low efficiency of PUFAs (Borges *et al.*, 2016).

6. Immunosensors

One significant obstacle to extracting oil from microalgae is successfully accessing the cell biomass.

Traditional extraction methods known as conventional extraction, release carbon, and have environmental problems. Alternative extraction methods can develop the industry of microalgae cultivation (Morales-Contreras *et al.*, 2022). Before extraction, microalgae biomass should be pre-treatment with cell wall disruption for measuring the efficiency of counted microscopic

cells. The formula used to calculate the yield of oil extracted efficiency: The unit of measurement for extracted lipid and dry microalga biomass is a milligram (mg).

Lipid extraction efficiency:

$$\frac{\text{Extracted lipids}}{\text{Dry microalgal biomass}} \times 100$$

Chemical methods include osmotic shock, acid or alkali treatment, and temperature shock can destroy significant products and biomolecules (Table 3). Some biological methods are also used, such as algicidal bacteria and enzyme treatments. Enzymes had less destruction to the products of cells. By contrast, algicidal bacteria reduce costs (Table 3).

Table 2.

Mechanical methods for extraction of EPA from oily microalga.

Microalgae	Extraction method	Cell disrupt methods	EPA yield (mg. g cell ⁻¹)	References
<i>Nannochloropsis gaditania</i>	Supercritical fluid (CO ₂)	Bead milling	11.5	(Molino <i>et al.</i> , 2019)
<i>Nannochloropsis</i> sp.	Hexane: isopropanol	High-pressure homogenization	34.4	(Bernaerts <i>et al.</i> , 2020)
<i>Nannochloropsis</i> sp.	Hexane	High shear mixer	PUFAs: 56.2	(Kwak <i>et al.</i> , 2020)
<i>Nannochloropsis</i> sp.	Brine solution	Microwave	10.42	(Zghaibi <i>et al.</i> , 2019)
<i>N. oceanica</i>	Ethanol	Ultrasonication	15.4	(Figueiredo <i>et al.</i> , 2019)

Table 3.

Extraction methods of lipids from oily microalga.

Microalgae	Extraction method	Lipid yield (mg. g cell ⁻¹)	References
<i>Nannochloropsis</i> sp.	Hexane (Chemical)	DS: 50.8	(Wu <i>et al.</i> , 2017)
<i>Nannochloropsis</i> sp.	Chloroform (Biological)	EPA: 19.97	(Wu <i>et al.</i> , 2017)
<i>N. oculata</i>	Hexane (Biological)	Lipids: 457	(Wang & Yuan, 2014)

6.1. Soxhlet

This method can use in industry and need temperature, and organic solvent (removed by rotary evaporation (He *et al.*, 2019). Extraction of lipids from microalgae with solvent could have high efficiency (Kim *et al.*, 2013). Studies on the

systems that rely on solvent showed that yield EPA content depends on microalgae species. Furthermore, the composition of polar and non-polar solvents causes increased product extraction efficiency (Mitra & Mishra, 2019).

6.2. Folch

Lipids were extracted with the chloroform-methanol solution in a water bath shaker and centrifuged (Folch *et al.*, 1957). The result indicates that when using *Isocheims* sp. biomass, in the folk extraction method efficiency of lipid extraction and the value of TFAS recovery is higher than in the Soxhlet method (He *et al.*, 2019).

6.3. Pulsed electric field (PEF)

This method results in an extraction called electroporation and increases the permeabilization cell. This process uses two electrodes in the milieu of the medium, caused transferred the electric charge inside and outside of cells.

The phenomenon of displacement of electric load leads to the creation of pores in the cell membrane and leads to the transpiration of the contents of microalgae that are inside the membrane of cells (Bodénès *et al.*, 2016).

Used solvent controller, diatomaceous earth, and solvents (ethanol) (He *et al.*, 2019). Using wet microalgae biomass causes thrift in production costs, time, and energy (Taher *et al.*, 2014). The extraction operation with a pulsed electric field is simple without environmental pollution (Zhou *et al.*, 2022).

6.4. Pressurized fluid extraction (PFE)

The PLE method, also called accelerated solvent extraction (ASE), is based on extraction at elevated temperature and pressure to maintain the solvent in liquid states for short periods. This extraction method causes to be extracted at a high speed of the extraction process and high efficiency and can be used to extract solid or semi-solid samples (Zhou *et al.*, 2022). In this method, environmentally friendly solvents such as water and ethanol are used (Castejón *et al.*, 2018).

6.5. Ultrasonic assisted

This method reduces the time of extraction and solvent consumption, and solvent could be penetration cell materials. Which, in turn, improves cell content release into a massive perimeter. Some limitations are high power

consumption, and scaling that up is difficult (Luque-Garcia & De Castro, 2003). Briefly, in the solvent, some bubbles are created by ultrasonic waves near the cell walls bubbles burst, which causes contractions (lipid) released into the solvent, because waves produce shock (Cravotto *et al.*, 2008; Wei *et al.*, 2008). Extraction with ethanol combined with pretreatment of the ultrasound led to enhanced efficiency of the EPA compound in *N. oceanic* microalgae (Figueiredo *et al.*, 2019).

6.6. Osmotic shock

Reduces osmotic pressure suddenly, cells burst and unhand their contents (Mario, 2010).

6.7. Supercritical fluid extraction (SFE)

This technique is utilized with CO₂ for lipid extraction from microalgae (Mercer & Armenta, 2011). Some advantages of this method are the use of low extraction temperature, as well as, lower environmental damage as no toxic solvent is used, and fast separation (Sahena *et al.*, 2009).

Similarly, some compounds had efficiencies instead of CO₂, such as sulfur, hexafluorides, pentane, butane, hexane, fluorinated hydrocarbons, and nitrogen (Adeoti & Hawboldt, 2014). By contrast, the performance of CO₂ is more traditional than the other compounds because it has a low cost for available. Water is distinguished from CO₂. In consequence, to prevent this, before extraction, biomass should be dry (Abdullah *et al.*, 2010).

6.8. Microwave

Microwave heating systems lose a little heat in their surroundings. The heating of the microwave is not direct and influences solvents and materials. If it is applied, the diminution of moisture in the microalgae cell wall leads the process to generate considerable pressure, which microalgae cell wall to break (Mercer & Armenta, 2011).

7. Calculation indices of quality of lipid

7.1. Atherogenic index (AI)

Demonstrates the correlation between total saturated fatty acids and unsaturated fatty acids (Ulbricht & Southgate, 1991).

$$(AI) = \frac{[(C12:0+(4 \times C14:0)+C16:0)]}{(\Sigma MUFA + \Sigma \omega-6 + \Sigma \omega-3)}$$

$\Sigma MUFA$ = sum of monounsaturated fatty acids, $\Sigma \omega-3$ = sum of omega-3 polyunsaturated fatty acids, $\Sigma \omega-6$ = sum of omega-6 polyunsaturated fatty acids, C16:0 = Palmitic acid, C14:0 = Myristic acid, C12:0 = Lauric acid.

7.2. Thrombogenic index (TI)

Terminating Input (TI) refers to a mechanism or a process used in various systems, where an input or a process is concluded or terminated after a specific condition is met. This condition could involve receiving a particular signal, data, or input, or it could be based on a predefined number of iterations or operations. In the context of computer science or programming, TI can be seen in functions or processes where the system automatically stops receiving input after completing a certain task, like reaching a time limit or meeting the required conditions. TI mechanisms are commonly used in scenarios like automated data processing systems, where once the input reaches a terminal state or condition, no further input is allowed.

n-3 PUFA are less anti-atherogenic than MUFA and n-6 PUFA (Ulbricht & Southgate, 1991).

$$(TI) = \frac{(C14:0 + C16:0 + C18:0)}{[(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n-6 + (3 \times \Sigma n-3) + (\frac{\Sigma n-3}{\Sigma n-6})]}$$

$\Sigma MUFA$ = sum of monounsaturated fatty acids, $\Sigma n-3$ = total omega-3 fatty acids, $\Sigma n-6$ = total omega-6 fatty acids, C18:0 = stearic acid, C16:0 = palmitic acid, C14:0 = Myristic acid.

7.3. Polyene index (PI)

Proportional-integral (PI) refers to a type of control system or algorithm commonly used in feedback control systems. It combines two control strategies: the Proportional component, which generates an output that is proportional to

the error (the difference between the desired setpoint and the actual value), and the Integral component, which addresses the accumulation of past errors over time. The PI controller works by adjusting the output based on both the current error (proportional term) and the historical sum of errors (integral term), effectively correcting both present and past discrepancies. PI controllers are often used in systems where minimizing steady-state errors is crucial, and they are widely applied in areas such as industrial automation, robotics, and temperature control systems (Lin *et al.*, 1995).

$$(PI) = \frac{EPA+DHA}{16:0}$$

6n-3= DHA, 5n-3= EPA, 16:0= palmitic acid

7.4 Referenced Findings and Data Interpretation

Studies indicate that replacing sea salt with sodium sulfate can boost DHA content and improve the cultivation yield in large-scale operations (Chen *et al.*, 2016). Similarly, using sodium sulfate as a nitrogen source in microalgae media has shown an increase in DHA production (Chalima *et al.*, 2020).

Key strategies to enhance EPA and DHA production in microalgae include:

- 1-Optimizing cultivation conditions to maximize EPA and DHA yield in biomass.
- 2-Selecting efficient strains with high biomass and lipid production capabilities; and
- 3-Exploring genetic modification techniques to naturally enhance EPA and DHA production (Song *et al.*, 2013; Zhou *et al.*, 2013). The amount of DHA is influenced by the lipid content of microalgae cells, lipid concentration, and cultivation period (De Swaaf *et al.*, 2003).

Closed-system photobioreactors offer an economical approach to PUFA production, although setup costs can be high. Utilizing carbon dioxide not only mitigates environmental pollution but also boosts lipid yield. For efficient extraction, a pretreatment extraction approach with solvents at high temperatures minimizes

solvent use and enhances productivity (Kim *et al.*, 2013). The ideal extraction method for EPA from *Nannochloropsis* species includes a combination of blue and red light, ultrasound, and ethanol solvent.

Figure 6 (below) illustrates the distribution of PUFAs across various microalgae strains, highlighting those strains like *Chlorella*, *Diatom*, *Chaetoceros*, and *Nannochloropsis* contain higher amounts of PUFAs, making them prime candidates for large-scale production.

As global PUFA demand rises, microalgae are increasingly cultivated on an industrial scale as a sustainable source of omega-3 for human and animal diets. However, despite its potential, large-scale cultivation remains cost intensive.

8. Conclusions and perspectives

Selection of an appropriate microalgae species is the first step for high yields of EPA and DHA. This is because the strain chosen can make or break the success of the production system, especially for large-scale outdoor cultivation. It has been investigated that PUFA content varies among strains, which may be considered when scaling up from small-scale to large-scale production. In practice, actual growth in marine microalgae could be totally different, considering the cultivation conditions, such as temperature limits in tropical and subtropical climates. There is also a balancing act between high lipid storage and fast growth rates for the optimization of productivity along with economic feasibility. Generally, efficient microalgae cultivation involves two important steps: upstream processes for optimum growth and downstream processes involved in lipid extraction and purification. Besides this, hybrid techniques of extraction involving solvent, temperature, and pressure have shown some promise in reducing costs with the least environmental impact. The key environmental parameters involve temperature, light, salinity, and nutrient level, which are very crucial to realize improvements in biomass productivity and yield of the lipid.

This will require the optimization of environmental controls to meet the rising global demand for this sustainable source of omega-3 fatty acids with new cultivation and extraction

methodology. In its commercial exploitation, one of the key issues to be addressed is the high cost associated with large-scale operations. Advanced methodologies integrated with the understanding of strain-specific characteristics hold tremendous potential for substantial improvement in the production of both EPA and DHA.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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