

Sản xuất các axit béo thiết yếu không bão hòa đa chuỗi dài bởi sinh trưởng quang tự dưỡng, hợp dưỡng và dị dưỡng ở các vi sinh vật quang hợp và không quang hợp: tổng quan

TÓM TẮT

Một số vi sinh vật quang hợp và không quang hợp như vi tảo, sinh vật nguyên sinh hoặc nấm, được biết đến như là nguồn sản xuất tự nhiên của các axit béo không bão hòa đa chuỗi dài (LC-PUFA). Trong đó, một số loài được biết đến như là những sinh vật dị dưỡng bắt buộc, hợp dưỡng hoặc quang tự dưỡng bắt buộc. Tuy nhiên, ngày càng có nhiều loài vi tảo, trước đây được biết là sinh vật quang tự dưỡng bắt buộc, nhưng nay được xác định là sinh vật hợp dưỡng hoặc dị dưỡng. Các con đường sinh tổng hợp và điều kiện nuôi cấy của các vi sinh vật này được so sánh để làm nổi bật các yếu tố ảnh hưởng đến quá trình sản xuất và phân phối LC-PUFA trong tế bào. Sản xuất LC-PUFA đã được cải thiện bằng cách lựa chọn quy trình nuôi cấy và chủng vi sinh vật. Các phân tích về sản lượng chuyển đổi và năng suất của LC-PUFA trong nuôi cấy quang tự dưỡng, hợp dưỡng và dị dưỡng làm sáng tỏ hiệu suất sản xuất LC-PUFA bởi các sinh vật quang hợp và không quang hợp.

Từ khóa: *Vi tảo, axit béo không bão hòa đa (PUFA), sinh trưởng quang tự dưỡng, sinh trưởng hợp dưỡng, sinh trưởng dị dưỡng.*

Photo-autotrophic, mixotrophic and heterotrophic production of essential long chain polyunsaturated fatty acids in photosynthetic and non-photosynthetic microorganisms: a review

ABSTRACT

Some photosynthetic and non-photosynthetic microorganisms as microalgae, stramenopiles or fungi, are known as natural producers of long chain polyunsaturated fatty acids (LC-PUFAs). Among those, some species are known as obligate heterotrophs, mixotrophs or obligate phototrophs. However, more and more microalgal species, previously reported as obligate photo-autotrophs, are now identified as mixotrophs or heterotrophs. The biosynthetic pathways and cultivation conditions of these microorganisms are compared to highlight the factors influencing production and distribution of LC-PUFAs in the cells. LC-PUFA production has been improved by the choice of cultivation processes and microorganism strains. Analyses of the conversion yields and productivities of LC-PUFAs in photo-autotrophic, mixotrophic and heterotrophic cultivation elucidate the performance of LC-PUFA production by photosynthetic and non-photosynthetic organisms.

Keywords: *microalgae, polyunsaturated fatty acid (PUFA), photoautotrophic growth, mixotrophic growth, heterotrophic growth.*

Abbreviations: *LC-PUFAs: long chain polyunsaturated fatty acids; GLA: gamma-linolenic acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; H: heterotrophic growth; M: mixotrophic growth; P: photoautotrophic growth; DO: dissolved oxygen; PKS: polyketide synthase; ROS: reactive oxygen species; TAG: triacylglycerol; MAG: monoacylglycerol; PC: phosphatidylcholine; PE: phosphatidylethanolamine; MGDG: monogalactosyldiacylglycerol; DPG: diphosphatidylglycerol; SQ: sulfoquinovosyldiglyceride; mg/L/d: milligram.liter⁻¹.day⁻¹.*

1. INTRODUCTION

During last decades, there was growing interest in supplying unsaturated lipids to animal and human.¹ Indeed, some particular unsaturated lipids have been shown to benefit to animal and human health.^{2,3} These lipids are provided either by food intake or by extracts e.g. fish oils, olive oils, soybean oils, canola oils, flaxseed oils.⁴ These sources are dependent on seasonal variations or the availability of natural resources. Moreover, fish oils are dependent on risks of contamination by xenobiotics^{5,6} and of unpleasant smell and taste. Therefore, these drawbacks were the reason why relatively recent bioprocesses for production by microorganisms in bioreactors have been developed.^{7,8} One of the first industrial applications was the DHA production by a microalga *Cryptocodinium cohnii*.⁹ Other photosynthetic and non-photosynthetic microorganisms were also found to produce unsaturated lipids.^{10,11}

The unsaturated lipids having 18 carbons or more in length with two or more double bonds were characterized as LC-PUFAs. Gamma-linolenic acid (GLA), arachidonic acid (ARA), eicosahexaenoic acid (EPA) and docosahexaenoic acid (DHA) are the essential LC-PUFAs which have been found in different sources as plant, fish, egg... In recent years, these essential LC-PUFAs have been produced by microorganisms.¹ These microorganisms include some microalgae, lower fungi, and bacteria which were grown in photoautotrophic, mixotrophic or heterotrophic condition (aerobic or anaerobic condition). These conditions also affect LC-PUFA content in the cells.¹² LC-PUFAs produced by microorganisms were naturally esterified as glycolipids, phospholipids, and neutral lipids which were constituted of membrane compositions.

Oxygen plays an important role for the most life on the earth because all higher organisms are aerobioses. Oxygen is indispensable for aerobioses. In cells, oxygen can transform into more reactive forms, Reactive Oxygen Species (ROS) which are toxic to cells. Under oxidative stresses, cells have to maintain the balance of the production between ROS and antioxidant enzymes. However, when the generation of ROS overtakes antioxidant enzymes, the damage of lipids, proteins and nucleic acids occurs.¹³ Besides the effects of cell component damage, oxygen also participates in biosynthesis and metabolism of cell compositions. During biosynthesis of LC-PUFAs, oxygen plays a role as an electron acceptor in reduction of fatty acids to form the double bonds.

In cultivation, some factors were useful for overproduction of LC-PUFAs such as temperature, pH, salinity, light.^{14,15} oxygen tension.¹⁶ Besides the environmental factors, nutritional factors also affect LC-PUFA biosynthesis. Organisms used in culture can be photoautotrophs, mixotrophs or heterotrophs. Photoautotrophs are organisms that obtain energy from light and carbon source from CO₂ to synthesize organic compounds in the cells while mixotrophs can use energy from light and carbon sources from organic compounds.¹⁷ Heterotrophs obtain energy and carbon sources from organic compounds.

For improvement of LC-PUFA production, many different types of bioreactors were used from pilot to industrial scales.^{8,18,19} Depending on the value of the desired products, photosynthetic production can be carried out in open systems and closed systems (photobioreactor).¹⁵ For heterotrophic culture, the classical enclosed bioreactors were used. Industrial reactors and data are available for some photoautotrophic and heterotrophic processes but a lesser point for mixotrophic process.

The objective of this review is to compare the performance of LC-PUFA production by photosynthetic and non-photosynthetic microorganisms.

2. MICROBIAL SOURCES OF LC-PUFA

LC-PUFAs are produced by various microorganisms, from prokaryotes to eucaryotes. They could be also classified either

also grow and produce GLA in 3 modes of nutrition.^{66,97}

as non-photosynthetic microorganisms or photosynthetic microorganisms (table 1).

Non-photosynthetic LC-PUFA producers constitute a large group of microorganisms that obtain energy and carbon from organic compounds. This group includes bacteria, fungi, fungus-like microorganisms and some microalgae. Some bacteria have been shown to produce EPA²⁰⁻²² or DHA.^{23,24} Some fungi can produce GLA, ARA or EPA. GLA-producing fungi include genera *Mortierella*,²⁵⁻²⁹ *Cunninghamella*³⁰⁻³⁴ and *Mucor*.³⁵⁻⁴¹ ARA-producing fungi include genera *Mortierella*⁴² and *Pythium*.⁴³ These fungal genera are also able to produce EPA.⁴⁴⁻⁴⁸ Other groups include fungus-like microorganisms, known as Stramenopiles, characterized as particularly marine stramenopilan protists belonging to the class Labyrinthulomycetes. They differ from fungi in composition of their cell walls (absence of chitin) and rhizoids.⁴⁹ Stramenopiles produce high levels of DHA up to 65.9% of total fatty acids.⁵⁰ The genera *Thraustochytrium*,⁵¹⁻⁵⁶ *Schizochytrium*⁵⁷⁻⁶⁰ and *Aurantiochytrium*^{61,62} are representatives for DHA production. Finally, the microalga *Cryptothecodinium* is included in the non-photosynthetic microorganisms, as it is an obligate heterotroph. This microalga can produce high amounts of DHA up to 63.2% of total fatty acids.⁶³

Photosynthetic microorganisms are constituted of the microorganisms for which energy is provided by light and carbon either by inorganic carbon (photoautotrophy) or by organic carbon sources (mixotrophy). This group includes cyanobacteria and microalgae. Some of them can grow mixotrophically and/or heterotrophically.⁶⁴⁻⁶⁷ Cyanobacterium *Spirulina* only biosynthesize GLA while some other microalgae can biosynthesize ARA, EPA or DHA. Not all cyanobacteria or microalgae species can produce LC-PUFAs, only certain cyanobacteria or microalgae can biosynthesize these LC-PUFAs. Microalgae can produce ARA as *Porphyridium*,⁶⁸ *Parietochloris*,⁶⁹⁻⁷¹ produce EPA as *Monodus*,⁷²⁻⁷⁵ *Porphyridium*,^{68,76} *Phaeodactylum*,^{64,65,77-79} *Nannochloropsis*,⁸³⁻⁸⁷ *Navicula*,^{88,89} *Nitzschia*,⁹⁰⁻⁹⁴ *Skeletonema*.⁹⁵ Some microalgae such as *Isochrysis* or *Pavlova* contain both EPA and DHA in their cells.⁹⁶ *Porphyridium* produces both ARA and EPA.⁶⁸ *Nannochloropsis*, *Nitzschia laevis* can produce EPA in 3 modes of nutrition.^{12,83} *Spirulina* can

Table 1. Distribution of LC-PUFAs in microorganisms.

Microorganisms		Strains (*)	GLA	ARA	EPA	DHA
Non-photosynthetic	Bacteria	<i>Shewanella oneidensis</i> ATCC 700550 ⁹⁸			+	
		<i>Moritella marina</i> ATCC 15381 ⁹⁹				+
	Fungi	<i>Cunninghamella echinulata</i> CCRC 31840 ³⁰	+			
		<i>Mortierella alpina</i> ATCC 16266 ¹⁰⁰		+		
		<i>Mortierella alpina</i> ATCC 32222 ⁴⁵			+	
	Fungus-like protists	<i>Thraustochytrium aureum</i> ATCC 34304 ⁵²				+
		<i>Thraustochytrium roseum</i> ATCC 28210 ⁵⁶				+
	Microalgae	<i>Cryptocodinium cohnii</i> ATCC 30772 ¹⁰¹⁻¹⁰³				+
Photosynthetic	Cyanobacteria	<i>Spirulina platensis</i> UTEX 1928 ⁶⁶	+			
	Microalgae	<i>Porphyridium cruentum</i> SAG 1380-1a ⁶⁸		+	+	
		<i>Phaeodactylum tricornutum</i> UTEX 640 ⁷⁸			+	
		<i>Isochrysis galbana</i> UTEX 987 ⁹⁶			+	+

(*) Representative strains associated with applied biotechnological studies.

3. OXYGEN AND LC-PUFA BIOSYNTHESIS

There are two different biosynthetic pathways of LC-PUFAs in microorganisms: anaerobic and aerobic pathways (figure 1).¹⁰⁴ This classification based on the oxygen dependence of PUFA biosynthesis reactions.

The term “anaerobic pathway” does not mean that the pathway only occurs in anaerobic

condition. It can operate in the presence of oxygen but oxygen was not used for formation of double bonds. The formation of double bonds in this pathway is carried out by a dehydration to remove a water molecule from hydroxyacyl-acyl carrier protein (ACP).

The anaerobic pathway was found in some non-photosynthetic microorganisms such as some bacteria and fungus-like microorganisms. LC-

PUFA biosynthesis in some bacteria is generally carried out by PKS-like system.^{98,105,106} To date, intermediates of this pathway have not yet been determined in detail. Some fungus-like microorganisms such as *Thraustochytrium* and *Schizochytrium* carried out two pathways for LC-PUFA biosynthesis: Polyketide Synthase (PKS) anaerobic pathway and aerobic pathway.^{107,108} Results of studies in *Cryptocodinium cohnii* indicated that DHA biosynthesis by this microalga was not catalyzed by the desaturases and elongases in aerobic pathway¹⁰⁹ but would be made by anaerobic PKS pathway.¹¹⁰

In aerobic pathway, molecular oxygen is required in biosynthesis of unsaturated fatty acids. It acts as an electron acceptor for double bond formation in the unsaturated fatty acids. This process is catalyzed by the desaturase enzymes which remove 2 H atoms from saturated or unsaturated fatty acids to form double bond in these fatty acids. These two H atoms combine with O atom of O₂ to form H₂O. The other O atom is reduced by cytochrome b₅ Fe²⁺.

The aerobic pathway occurs in microorganisms such as cyanobacteria, fungi and microalgae. Desaturases use molecular oxygen to form double bonds for unsaturated fatty acids production. This biosynthetic pathway has been studied in photosynthetic microorganisms by using ¹⁴C labelled intermediates. In the red microalga *Porphyridium cruentum*, it was shown that externally supplied unsaturated fatty acids were assimilated in mixotrophic cultures. Two routes of EPA biosynthesis from linoleic acid (C18:2, n-6) precursors have been suggested: one route along n-6 pathway and another along n-3 pathway.¹¹¹ In diatom *Phaeodactylum tricornutum*, four routes of EPA biosynthesis

were revealed by use the radiolabeled intermediates. Two routes pass along n-3 pathway, one route pass along n-6 pathway and other route pass along both n-3 and n-6 fatty acids as intermediates.¹¹² In *Parietochloris incisa*, ARA biosynthesis is carried out in cytoplasm and in chloroplast.¹¹³ In *Pavlova* and *Isochrysis*, DHA was synthesized by aerobic pathway. Gene *IgASE1* which encoded an elongating enzyme in aerobic pathway was identified in *Isochrysis galbana* and expressed in yeast.^{114,115} In addition, the genes *pavELO* (*Pavlova*), which catalysed conversion of EPA into DPA and *IgD4* (*Isochrysis*) catalysed conversion of DPA into DHA, were also identified and transformed into yeast cells. The yeast cells were cultured with exogenously supplied EPA and they can synthesize DHA from EPA.¹¹⁶ Many desaturase and elongase genes in *Mortierella alpina* have been isolated and characterized.¹¹⁷ This indicated that PUFA biosynthesis in this fungus is carried out by aerobic pathway. Other proof showed that when using SAN 9785, the inhibitor of desaturases, GLA content in the cells of *Spirulina* was significantly influenced.⁷⁶

Using intermediates of the biosynthetic pathways seems to increase the content of LC-PUFAs. The ARA content of *Porphyridium* was 23.2% of total fatty acids after addition of linoleate to the culture medium compared with 16.8% in the control.¹¹¹ Similarly, GLA content was 36.0% of total fatty acids when *Spirulina* was grown with linoleate, against 20.4% in the control.¹¹⁸ In the culture of *Mortierella* for EPA production, linseed oil contains α -linolenic acid (precursor for EPA biosynthesis) as a major fatty acid (58%) was supplemented in the medium, resulted in an increase of EPA content.⁴⁵

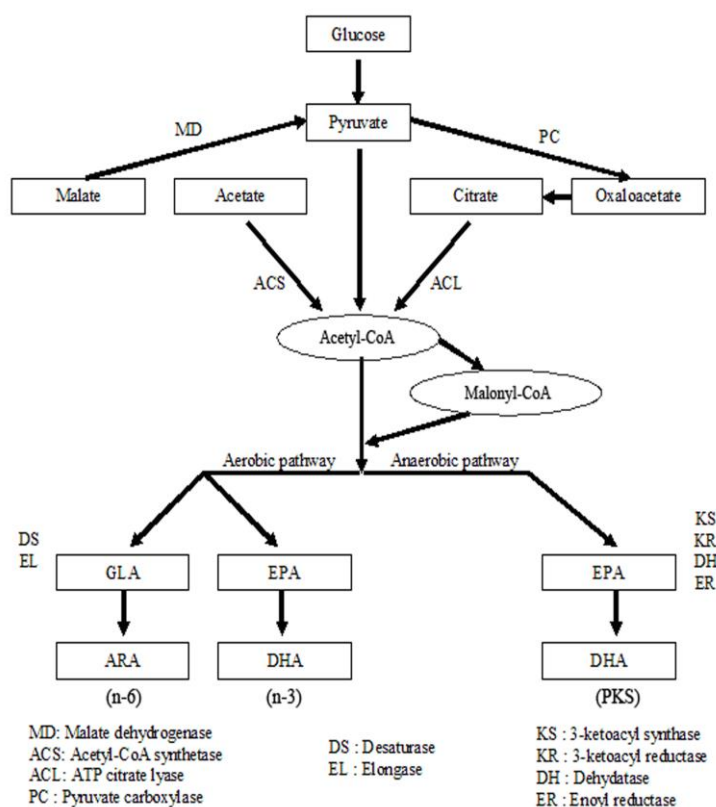


Figure 1. Pathway of LC-PUFA biosynthesis.

4. FACTORS AFFECTING LC-PUFA PRODUCTION

4.1. Nutritional factors

4.1.1. Carbon sources

Carbon constitutes from 49 to 57% of biomass and about 80% of the LC-PUFAs, provided either by inorganic forms such as CO_2 or HCO_3^- for carbon skeletons in photoautotrophic growth or by organic forms as energy sources and carbon skeletons in mixotrophic or heterotrophic growth.

Glucose is the most commonly used carbon source for heterotrophic growth of microorganisms. However, the suitable carbon sources for growth and LC-PUFA production would be different among microorganisms. Starch and maltose were the suitable carbon sources for DHA production by *Thraustochytrium aureum* ATCC 34304.⁵² Starch was also the preferred carbon source for GLA production by *Cunninghamella echinulata* CCRC 31840³⁰ or for ARA production by *Mortierella alpina* ATCC 32222,²⁷ whereas glucose was optimal for DHA production by *Thraustochytrium* sp. KK17-3.¹¹⁹ Glucose was also the suitable carbon source for growth and EPA production by *Pythium irregulare* ATCC 10951⁴⁸ or for GLA production by *Mucor hiemalis* M4.¹²⁰ Glucose and starch were suitable

carbon sources for ARA production by *Mortierella alliacea* YN-15.¹²¹ Although ARA yield was highest with glycerol, ARA content in lipids was quite low. Thus, glucose was the best choice for ARA production by *Mortierella alpina* ATCC 16266.¹⁰⁰ In *Mortierella alpina* CBS 528.72, glucose gave the optimal growth and total lipid content but rhamnose gave a higher ARA content in total fatty acids.¹²² The complex sources as rice bran, wheat bran, peanut meal, sweet potato, linseed oil, soybean oil... were also investigated for ARA production by *Mortierella alpina* ATCC 32222.²⁷ Marine microalga *Cryptocodinium cohnii* could utilize acetic acid,¹²³ ethanol,¹⁰² carob pulp¹²⁴ for DHA production.

Some microalgae as *Nitzschia laevis* UTEX 2047 could mixotrophically grow with glucose¹² or *Nannochloropsis* sp. could utilize glucose or ethanol⁸³ for EPA production. *Phaeodactylum tricornutum* UTEX-640 could also grow and produce EPA in mixotrophic conditions with various carbon sources. Glycerol was found as the most suitable carbon source for growth and EPA production by this microalga.^{64,65,77} *Navicula saprophila* could grow with acetate in mixotrophic condition.^{88,89}

In photoautotrophic culture of *Phaeodactylum tricornutum* UTEX 640, the different concentrations of CO_2 were examined for

growth and EPA production. The optimal biomass and EPA yield were 2.5 g/L and 87.5 mg/L, respectively, obtained at 1% CO₂.⁷⁸ In the 4-day cultivation of *Nannochloropsis* sp., 2% CO₂ was supplied 12 h prior to the end of the exponential growth gave the highest EPA yield and productivity which were 340 µg/L and 126 µg/L/d, respectively. This productivity was twice as high as that in ambient air.⁸⁰ The elevation of CO₂ concentration (350 to 2800 µl/l CO₂) in photoautotrophic culture resulted in an increase of EPA content (21.9% to 25.3% of total fatty acids) in *Nannochloropsis* sp.^{81,125} *Monodus subterraneus* UTEX 151 was cultured at two different concentrations of CO₂ (1% and 5%). EPA content in total fatty acids obtained at 1% CO₂ was higher than that obtained at 5% CO₂.⁷²

4.1.2. Nitrogen sources - nitrogen starvation

Nitrogen constitutes from 8 to 12% of biomass. LC-PUFA producing microorganisms can grow on organic or inorganic nitrogen sources. *Thraustochytrium aureum* ATCC 34304 could utilize organic nitrogen sources as tryptone, peptone, malt extract, yeast extract and sodium glutamate. Cells were grown with yeast extract gave 5.0 g/L biomass and 247.7 mg/L DHA yield while those were 3.8 g/L (biomass) and 269.6 mg/L (highest DHA yield) with sodium glutamate.⁵² Tryptone was the most suitable for DHA production by *Thraustochytrium* sp. KK17-3 with 232.8 mg/L DHA.¹¹⁹ *Mortierella alpina* LPM 301 was grown in the medium with urea or potassium nitrate as nitrogen sources for ARA production. ARA yield was 4.5 g/L after 189 h of cultivation with potassium nitrate and 4.2 g/L after 210 h with urea.¹²⁶ Some other strains of *Mortierella* fungi have been studied with the inorganic and organic nitrogen sources. Yeast extract was found as the best nitrogen source for growth and ARA production by *Mortierella alpina*.^{122,127} Furthermore, combination of soluble starch 120% and the mixture (2:1, w/w) of KNO₃ and yeast extract were the best nitrogen sources for ARA production by *Mortierella alpina* ATCC 32222.²⁷ Ammonium hydroxide was used in the culture of *Mortierella alpina* DSA-12 as the nitrogen source and pH control.¹²⁸ Ammonium nitrate was found as suitable nitrogen source for GLA production by *Cunninghamella echinulata*.³⁰ *Nitzschia laevis* UTEX 2047 was heterotrophically cultivated with glucose. Nitrate, ammonium and urea were investigated for growth and EPA production. Biomass and EPA yield were over 4 g/L and 90 mg/L,

respectively, obtained with nitrate or urea but only 1.24 g/L biomass and 21.58 mg/L EPA with ammonium.⁹¹ Combination by the ratio 32:1 of glucose and mixture (1:2.6:1.3) of nitrate, tryptone and yeast extract was optimal for EPA production by *Nitzschia laevis* UTEX 2047.⁹⁴

Mixotrophic cultivation was carried out with *Phaeodactylum tricornutum* UTEX 640 in the presence of glycerol with urea or nitrate. The best results were 1.52 g/L/d biomass and 43.13 mg/L/d EPA obtained with 0.01 M urea in fed-batch.⁶⁵

In photoautotrophic cultivation of *Phaeodactylum tricornutum* UTEX 640, urea was the optimal nitrogen sources for EPA production.⁷⁸ Nitrate, nitrite and urea were utilized in the culture of *Isochrysis galbana*. DHA content in total fatty acids was highest (14.13%) obtained with urea at early stationary phase.¹²⁹ (NH₄)₂HPO₄ was the suitable source of nitrogen for growth and GLA production by *Spirulina platensis*.¹³⁰

Isochrysis galbana CCAP 927/1 was cultivated in nitrate starvation, DHA content in total fatty acids increased from 1.19 to 4.52% from 2nd day to 5th day of cultivation.¹³¹ Nitrogen starvation induced an increase in ARA content over 60% of total fatty acids in *Parietochloris incisa*.⁷⁰

4.1.3. Phosphorus sources - phosphorus starvation

Phosphorus participates in the energy transfer within cells and constitutes about 5-6% of phospholipids.

In fungus *Pythium irregulare* ATCC 10951, optimal EPA production (about 31 mg/L) was obtained at 0-3 mM phosphate. The increase of phosphate concentrations (6 – 24 mM) resulted in a decrease of EPA yield.⁴⁸

Effects of phosphate (0.05-0.5 g/L) were also examined on the growth and EPA production by *Phaeodactylum tricornutum* UTEX 640. Little change in biomass was observed in this range of phosphate concentrations but EPA yield was higher at phosphate levels of 0.1 – 0.5 g/L.⁷⁸

Phosphorus starvation was studied by 7-day cultivation of *Phaeodactylum tricornutum* in the phosphorus-deficient medium (no phosphate was added). A comparative control was made in parallel with 6.9 mg/L NaH₂PO₄. Results indicated that EPA content in total fatty acids decreased from 26.8% to 6.9% in the condition of phosphorus deficiency.¹³² Other study showed

that EPA content decreased from 28.2 to 15.5% mol of fatty acids when decreasing phosphate concentration (K_2HPO_4) from 175 to 0 μM in the 4-day cultivation of *Monodus subterraneus*.¹³³

4.1.4. Silicate

Silicate is an essential nutrient for diatom growth because cells need silicate to form their frustules. *Nitzschia laevis* UTEX 2047 was heterotrophically grown with glucose and silicate. The highest EPA yield (131 mg/L) was obtained at 20 g/L glucose and 32 mg/L silicate while the highest EPA productivity was 15.1 mg/L/d at 20 g/L glucose and 64 mg/L silicate.⁹⁰

In photoautotrophic conditions, the range of silicate from 8.8 – 176 μM has been examined for EPA production in the culture of *Nitzschia inconspicua*. Results showed that there was not significantly change in EPA content (about 4.0% of total fatty acids) and EPA yield (about 0.2 mg/L).¹³⁴ Similarly, the photoautotrophic growth of *Phaeodactylum tricorutum* was not significantly different in the levels of 0 to 50 mg/L silicate. Increase of silicate levels from 50 to 500 mg/L resulted in reducing growth (2.6 to 1.8 g/L biomass) and EPA content (72.5 to 35.0 mg/L EPA).⁷⁸

4.2. Environmental factors

4.2.1. Temperature

Optimal temperature for growth is often different from optimal temperature for LC-PUFA accumulation. The increase of LC-PUFA contents at low temperature is attributed to the cells maintain fluidity of membranes by biosynthesizing more LC-PUFAs.

Effect of temperature on production of ARA and EPA was studied in *Pythium ultimum*. The optimal temperature for ARA and EPA production was 25° C.⁴³ This temperature was also found as the most suitable temperature for ARA accumulation in *Mortierella alpina*^{122,127} and *Thraustochytrium roseum* ATCC 28210 for DHA production.⁵⁵ Highest DHA content in total fatty acids was found when *Schizochytrium limacinum* OUC88 was cultured at 16 – 23° C.¹³⁵ In *Aurantiochytrium* sp. strain mh0186, cells grew well at 15 – 30° C, but weakly at 10° C. The amount of DHA in total fatty acids was highest at 10° C. The DHA yield was similar at 15 – 30° C and was significantly higher than those at 10 and 35° C.¹³⁶

A range of temperature from 10 to 30° C was investigated in culture of *Phaeodactylum*

tricorutum 2038. Growth was inhibited at 30° C, slow at 25° C and optimal at 20° C. EPA content in dry weight was highest at 10° C.¹³⁷ However, optimal temperature for biomass and EPA production by *Phaeodactylum tricorutum* UTEX 640 was found at 21.5 – 23° C in the study of Yongmanitchai and Ward (1991).⁷⁸ The effect of temperature on GLA content was also studied in *Spirulina platensis* UTEX 1928. The suitable temperature for GLA accumulation was from 25 to 33° C.¹³⁸ The optimal EPA production was obtained at 8° C in the culture of *Porphyridium purpureum* 1380-1b.¹³⁹

4.2.2. pH

Generally, heterotrophic cultures were related to acid pH conditions. pH in the range of 5.5 – 6.5 was suitable for biomass and ARA production by *Mortierella alpina*. Maximal ARA content in total fatty acids was obtained at initial pH 6.5.¹²² ARA yield was highest at initial pH 6.0.^{100,140} Initial pH 6 was also favourable for DHA production by *Thraustochytrium*.^{52,55} Optimal growth and EPA production were obtained at initial pH 6 – 7 in culture of *Pythium irregulare*.⁴⁸ The highest DHA content in total fatty acids was 56.8% at initial pH 7.2 in *Cryptheconidium cohnii* ATCC 30556.¹⁴¹

Yongmanitchai and Ward have found the maximal EPA yield (93.1 mg/L) at initial pH 7.6 in the photoautotrophic culture of *Phaeodactylum tricorutum* UTEX 640.⁷⁸ The range of pH from 5.0 to 8.5 was tested for EPA production in *Porphyridium purpureum* 1380-1b. The highest EPA yield (1.79 mg/L) was obtained at pH 7.6.¹³⁹

4.2.3. Salinity

Some studies relating to effects of salinity on growth and LC-PUFA production have been investigated. A wide tolerance to salinity was found in *Schizochytrium limacinum* when this fungus-like microorganism was cultured in the salinity range from 0% to 200% that of seawater. In the optimal range of salinity for growth (50 – 200% of seawater), there was little change in dry cell weight. Although this strain could grow at 0% of salinity, the growth was lower than those at the optimal range of salinity.⁶⁰ *Thraustochytrium* sp. showed a slight resistance to high salinity, up to 200% that of seawater. The optimal salinity for growth and DHA production was 75% that of seawater.¹¹⁹ Sea salt from 2 – 50 g/L was also examined for growth and DHA production by *Thraustochytrium* sp. The highest biomass (24.7 g/L) and DHA yield (4.6 g/L) were obtained at 2 g/L NaCl.⁵³ The

optimal concentration of NaCl for DHA production by *Cryptocodinium cohnii* ATCC 30556 was 9 g/L.¹⁴²

In *Pavlova lutheri* SMBA 60, NaCl concentrations from 5 to 45 g/L were examined for EPA and DHA production. The highest EPA (about 4.7 mg/L) and DHA yield (about 2.6 mg/L) were obtained at 5 – 15 g/L NaCl.¹⁴³ *Phaeodactylum tricornutum* UTEX 640 gave the highest EPA yield at 0 – 10 g/L NaCl.⁷⁸ *Spirulina* was cultivated in the range 0 – 3.5 g/L NaCl, GLA content increased as NaCl level was raised to 0.6 g/L and then it decreased. GLA yield was highest (27 µg/ml) at 0.2 g/L NaCl concentration.¹⁴⁴

4.2.4. Light

Light also stimulates the growth and DHA production in *Thraustochytrium aureum*. Biomass and DHA yield in light exposed cultures were 70.4 g/L and 269.6 mg/L, respectively, higher than those in dark cultures.⁵² After that, some cultivations of *Thraustochytrium* for DHA production were carried out under light by other authors.^{55,145,146}

Light affects growth and fatty acids composition of microorganisms, especially the photosynthetic ones. *Pavlova lutheri* SMBA 60 was grown in semi-continuous cultures at the different light intensities 9, 19 and 30 W/m². The highest EPA and DHA productivities were obtained at 19 W/m².¹⁴⁷ Effects of intensities and photoperiods on fatty acid production by *Isochrysis galbana* have also been studied.¹⁴⁸ Percentage of EPA in total fatty acids and in dry weight were 35.7% and 4.4%, respectively when *Monodus subterraneus* was grown at 90 µmol photon/m²/s, which was higher than those at 170 µmol photon/m²/s.⁷² GLA content in total fatty acids of *Spirulina platensis* increased from 31.1 to 36.0% when increased the light intensity from 860 to 1400 µmol photon/m²/s.¹³⁸

4.2.5. Culture age

Effect of culture age on ARA production by *Mortierella alpina* I49-N₁₈ was investigated. ARA yield increased and was maximal at the 6th day, and then decreased.¹²⁷ The GLA yield was also maximal after 5 – 6 days in the culture of *Cunninghamella echinulata* CCRC 31840.³⁰

In photoautotrophic culture of *Pavlova viridis*, EPA and DHA content in late exponential phase (4 days) were 22.1 and 3.5 mg/g biomass, respectively, and decreased in linear phase (7 days) and stationary phase (13 days).¹⁴⁹

4.2.6. Dissolved oxygen

Oxygen atom constitutes from 27 to 32% of biomass and about 10% of the LC-PUFAs. The levels of DO affected growth and LC-PUFA production in heterotrophic culture of various microorganisms. *Cryptocodinium cohnii* gave higher DHA yield when cultured at DO of 10 – 50% of air saturation level.¹⁶ In *Schizochytrium limacinum* SR21, the culture was carried out in two stages, the first stage for biomass production where concentration of dissolved oxygen at 50% whereas at 10% for DHA production in the second stage.⁵⁷ DHA content of total fatty acids was 30.6% and 40% at 40% DO and 5% DO, respectively.¹⁵⁰ *Mucor rouxii* ATCC 24905 was shifted from anaerobic to aerobic conditions resulted in an increase of biomass and fatty acid content. Oxygen induced the expression of Δ^9 -, Δ^{12} - and Δ^6 - desaturase genes resulted in an increase of unsaturated fatty acids.¹⁵¹

5. DISTRIBUTION OF LC-PUFAS IN LIPID CLASSES

Distribution of LC-PUFAs in lipid classes is various among microorganisms. The nutritional and environmental factors affect the distribution of LC-PUFAs in the cells. Information on LC-PUFA localization in the lipid classes is determinant for the purification process.

In *Cunninghamella echinulata* ATHUM 4411, GLA distribution depended on developmental stages. GLA content in PC remained over 20% of total fatty acids in mid exponential, late exponential and stationary phase whereas that was changed in other lipid classes. ARA content in dry weight increased in non-polar lipids but decreased in polar lipids through growth phases.³² The distribution of LC-PUFAs in lipid classes in *Mortierella alpina* SC9 was influenced by salinity. TAG was the dominant lipid class of the cells (261.16 mg/g) which contained the highest proportion of ARA (30.29% of total fatty acids). When the cells were cultured at 20 g/L NaCl, TAG content increased 296.55 mg/g but ARA content decreased 21.24%.¹⁵² In *Nitzschia laevis* UTEX 2047, neutral lipids (78.6%) were the major component of the total lipids, in which TAG was the predominant component (87.9%) of neutral lipids. EPA was present 37.44% in TAG, 22.49% in MAG and 15.91% in PC.¹⁵³ EPA content increased in polar lipids but decreased in neutral lipids at 10 – 30 g/L NaCl.¹⁵⁴ When increasing the temperature from 15 to 23° C, EPA content slightly decreased in TAG but increased in glycolipids. EPA content in

phospholipids at 19° C was higher than that at 15° C and 23° C.¹⁵⁵ In *Schizochytrium mangrovei* FB3, TAG was the predominant component with 97.2% of neutral lipids. Neutral lipids constitute 95.9% of total lipids. PC was the major polar lipids which accounted for 47.78% of phospholipids. DHA was found as the main polyunsaturated fatty acid since it was 29.74% in TAG and 39.61% in PC.¹⁵⁶ PC in *Cryptocodinium cohnii* was the major component (63.6%) of polar lipids in which 57.2% were DHA.¹⁵⁷ However, it was stated that DHA accumulated predominately in *Cryptocodinium cohnii* cells as TAG, the neutral lipid fraction.⁹

Navicula saprophila was mixotrophically grown with acetate in which PC was the major component (55.7% of lipids) and EPA was concentrated 28.2% in PC whereas PC was only 47.9% of lipids and EPA was 19.0% of PC in photoautotrophic culture.⁸⁹

In photoautotrophic culture of *Parietochloris incisa*, TAG was the dominant lipid with 42.9% of fatty acids in logarithmic phase and 77% in stationary phase. ARA was mainly present in TAG with 43% in logarithmic phase and 47% in stationary phase.⁶⁹ Under nitrogen starvation, neutral lipids and ARA content in neutral lipids were 86.8% and 63.7% of total fatty acids compared to 62.1% and 50.8% in the control, respectively.⁷⁰ In *Pavlova lutheri*, TAG was the major component of nonpolar lipids and MGDG was the main component of polar lipids. EPA was present 45% in MGDG and 33% in TAG.

DHA was distributed 27% in TAG, 22% in DPG and 21% in betaine lipids.¹⁵⁸ Light affected distribution of EPA in lipid classes. Under low light intensity (9 W/m²), EPA accumulated in polar lipids was higher than that in non-polar lipids whereas it was conversely when cultured at 19 and 30 W/m². At these conditions of light, DHA content in non-polar lipids was higher than that in polar lipids. When increasing light intensity from 9 to 30 W/m², EPA and DHA contents in polar lipids decreased while EPA and DHA contents in non-polar lipids at 19 W/m² were higher than those at 9 and 30 W/m².¹⁴⁷ Galactolipid fraction contained 92% GLA in *Spirulina platensis* 2340.¹⁵⁹ Nitrogen starvation affected distribution of ARA and EPA in *Porphyridium cruentum*. ARA content in total fatty acids increased from 19.9% to 30.7% in the neutral lipids and from 46.3% to 61.2% in PC whereas EPA decreased from 43.2% to 16.9% in MGDG, 29.4% to 8.6% in SQ and 17.4% to 2.9% in PC.⁶⁸

Formation of lipid bodies was revealed by using fluorescent staining of endoplasmic reticulum (ER). Lipid bodies surrounded ER in oleaginous fungus *Mortierella ramanniana* IFO 8187.¹⁶⁰ The same result was observed in *Schizochytrium limacinum* SR21. The lipid bodies often contact with ER in all stages of the cells.¹⁶¹

In the photosynthetic microorganisms, the lipid body formation occurred in the inner thylakoid spaces of the chloroplast structure in *Isochrysis*^{162,163} or *Monodus subterraneus* UTEX 151.⁷⁴

Table 2. Distribution of LC-PUFAs in lipid classes.

Microorganisms	Modes of nutrition	LC-PUFAs	LC-PUFAs (mg/g dry weight)	
			Polar lipids	Non polar lipids
<i>Cunninghamella echinulate</i> ATHUM 4411 ³² (a)	H	GLA	0.93	7.04
<i>Mortierella alpine</i> SC9 ¹⁵² (b)	H	ARA	1.22	84.95
<i>Nitzschia laevis</i> UTEX 2047 ¹⁵⁴ (c)	H	EPA	9.81	61.19
<i>Nitzschia laevis</i> UTEX 2047 ¹⁵³	H	EPA	5.87	9.11
<i>Schizochytrium mangrovei</i> FB3 ¹⁵⁶	H	DHA	9.00	193.17

<i>Pavlova lutheri</i> SMBA 60 ¹⁵⁸	P	EPA	8.41*	4.95*
<i>Pavlova lutheri</i> SMBA 60 ¹⁵⁸	P	DHA	6.39*	2.26*
<i>Pavlova lutheri</i> SMBA 60 ¹⁴⁷ (d)	P	EPA	526.94*	170.77*
<i>Pavlova lutheri</i> SMBA 60 ¹⁴⁷ (d)	P	DHA	69.77*	177.99*

* unit: mg/g ash free dry weight (AFDW); (a) late exponential phase; (b) 0 % NaCl; (c) 10 g/L NaCl; (d) 9 W/m²

6. LC-PUFA YIELD AND PRODUCTIVITY OF PHOTOSYNTHETIC AND NON-PHOTOSYNTHETIC MICROORGANISMS

Non-photosynthetic microorganisms only grow and produce LC-PUFAs in heterotrophic condition while photosynthetic microorganisms

can grow and produce LC-PUFAs in photoautotrophic, mixotrophic and heterotrophic conditions (table 4). The LC-PUFA producers for high productivity have been selected to compare the performance of their production (table 3).

Table 3. Comparison of LC-PUFA yield and productivity of selected microorganisms.

LC-PUFAs	Microorganisms	Modes of nutrition	Biomass (g/L)	LC-PUFA yield (g/L)	LC-PUFA productivity (mg/L/d)
GLA	<i>Mucor rouxii</i> CBS 416.77 ⁴⁰	H	24.0	0.532	336.0
	<i>Cunninghamella echinulata</i> CCRC 31840 ³¹	H	38.1	1.349	269.8
	<i>Mortierella ramanniana</i> CBS 112.08 ²⁶	H	12.0	0.451	112.8
	<i>Spirulina platensis</i> M2 ³¹	P	-	-	26.4
ARA	<i>Mortierella alpina</i> DSA-12 ¹²⁸	H	72.5	18.800	1504.0
	<i>Mortierella alpina</i> ME-1 ¹⁶⁴	H	39.8	19.020	3396.4
	<i>Parietochloris incisa</i> comb. nov ¹⁶⁵	P	21.0	2.667	70.2
EPA	<i>Mortierella alpina</i> 20-17 ¹⁶⁶	H	24.5	1.350	103.8
	<i>Nitzschia laevis</i> UTEX 2047 ⁹²	H	-	-	174.6
	<i>Phaeodactylum tricornutum</i> UTEX 640 ⁶⁵	M	15.4	0.436	43.1

	<i>Phaeodactylum tricornutum</i> UTEX 640 ¹⁶⁷	M	-	-	56.0
	<i>Phaeodactylum tricornutum</i> UTEX 640 ⁷⁹	P	1.7	0.083	25.1
	<i>Monodus subterraneus</i> UTEX 151 ¹⁶⁸	P	-	-	58.9
DHA	<i>Cryptocodinium cohnii</i> ATCC 30772 ¹⁰²	H	109	11.700	1276.4
	<i>Schizochytrium limacinum</i> ATCC 1381 ⁵⁹	H	48.1	13.300	3325.0
	<i>Schizochytrium</i> ¹⁵⁰	H	178	33.286	16560.0
	<i>Isochrysis galbana</i> UTEX 2307 ¹⁶⁹	P	-	-	4.3

6.1. Heterotrophic production

Until now, numerous data of heterotrophic LC-PUFA production by non-photosynthetic microorganisms have published.

For GLA production, fungi were found as producers in high GLA productivity. *Mucor rouxii* CBS 416.77 was cultivated with glucose and Difco yeast extract. Biomass and GLA productivity was 24 g/L and 336 mg/L/d, respectively.⁴⁰

Higashiyama *et al.* has compared productivity of ARA production by *Mortierella*, in which strain *Mortierella alpina* 1S-4 gave high ARA productivity (1300 mg/L/d).⁴² However, Hwang *et al.* cultivated *Mortierella alpina* DSA-12 in fed-batch by using NH₄OH as a nitrogen source and pH control which obtained 1504 mg/L/d ARA, higher than former productivity in the culture of Higashiyama *et al.*¹²⁸ *Mortierella alpina* ME-1 was a UV-mutant of ATCC 16266 gave 19020 mg/L ARA at 5.6 days which was highest found in the reports.¹⁶⁴

EPA production has been reviewed by Bajpai and Bajpai.¹⁴ High EPA yield and productivity were 1350 mg/L and 103.8 mg/L/d in the culture of *Mortierella alpina* 20-17.¹⁶⁶ *Nitzschia laevis* UTEX 2047 was grown in perfusion culture with cell bleeding. EPA productivity obtained in this cultivation (174.6 mg/L/d) was highest EPA productivity found.⁹²

Stramenopiles was utilized as DHA producing microorganisms. DHA yield and productivity

have been compared among various strains. *Schizochytrium limacinum* SR21 gave the highest DHA yield and productivity with 13300 mg/L and 138 mg/L/h DHA.¹⁷⁰⁻¹⁷² The other strain of *Schizochytrium* which has been studied by Bailey *et al.* produced a very high concentration of DHA 23.45 g/L in 42 h.¹⁵⁰ *Cryptocodinium cohnii* was also a DHA producing microalga. The parameters of culture and DHA production were collected in the review of Mendes *et al.*⁹ The fed-batch cultivation on ethanol produced 11700 mg/L DHA in 220 h was the highest productivity in this microalga.¹⁰²

6.2. Mixotrophic production

Mixotrophic production was found in photoautotrophic microorganisms which have growth capacity with organic compounds under light. Up to now, most of LC-PUFA producing microorganisms in mixotrophic condition were EPA producing microalgae and GLA producing cyanobacteria (table 4). *Phaeodactylum tricornutum* UTEX-640 was cultivated with carbon sources to evaluate growth and EPA production. Glycerol was found as the most suitable source of carbon.^{65,77} Fed-batch culture with 0.1 M concentration of glycerol and the successive additions of ammonium chloride gave 16.2 g/L biomass concentration, 61.5 mg/L/h biomass productivity and 33.5 mg/L/d EPA productivity. This EPA productivity was 10-fold greater than the maximum productivity obtained in the photoautotrophic control

culture.⁶⁴ Additionally, fed-batch with glycerol and urea gave 43.13 mg/L/d EPA productivity which was 13-fold higher than the maximum EPA productivity obtained in photoautotrophic culture of the control.⁶⁵ Other result of *Phaeodactylum tricornutum* UTEX-640 indicated that EPA productivity (56 mg/L/d) in mixotrophic culture (with glycerol) was approximately 3-fold higher than that in photoautotrophic culture.¹⁶⁷ *Navicula saprophila* was mixotrophically cultivated with acetate. EPA content obtained in this condition was 19.2 mg/g biomass that was higher than those obtained in photoautotrophic and heterotrophic conditions.⁸⁸ EPA content in biomass was 34.6 mg/g when *Navicula saprophila* was cultured with 2 mM acetate and 2% CO₂.⁸⁹ Performance of EPA production in three nutritional modes

was compared in the culture of *Nitzschia laevis* UTEX 2047. Growth and EPA production were highest in mixotrophic culture. EPA yield and productivity were 52.32 mg/L and 10.46 mg/L/d, respectively.¹² *Nannochloropsis* sp. also showed that they can grow and produce EPA in 3 nutritional modes. Glucose and ethanol were utilized as carbon sources for EPA production which gave 23.4 mg/L and 23.0 mg/L EPA, respectively, in mixotrophic cultivation after 8 days.⁸³ An increase of EPA yield up to 56 mg/L was obtained in 10 days of fed-batch culture with an addition of glucose and nitrate.⁸² *Spirulina platensis* KCTC AG20590 was mixotrophically cultivated with the long or short chain carbon sources. Results indicated that GLA content increased when compared with the control.⁹⁷

Table 4. Comparison of LC-PUFA productivity between the nutritional modes.

Strains	LC-PUFAs	LC-PUFA productivity (mg/L/d)		
		PA	M	H
<i>Nannochloropsis</i> sp. ⁸³	EPA	3.13	3.34	1.44
<i>Nitzschia laevis</i> UTEX 2047 ¹²	EPA	3.39	10.46	6.37
<i>Phaeodactylum tricornutum</i> UTEX-640 ⁶⁵	EPA	3.35	43.13	-
<i>Phaeodactylum tricornutum</i> UTEX-640 ¹⁶⁷	EPA	18.0	56.0	-
<i>Navicula saprophila</i> ⁸⁹	EPA	4.93	14.8	-
<i>Spirulina platensis</i> KCTC AG20590 ⁹⁷	GLA	0.43	1.70	-

6.3. Photoautotrophic production

Photoautotrophic production was only found in photosynthetic microorganisms. Table 5 presented performance of essential LC-PUFAs from photosynthetic microorganisms.

GLA productivity was 26.4 mg/L/d obtained from *Spirulina platensis* M2 in outdoor culture.¹⁶⁸

Parietochloris and *Porphyridium* were ARA-producing microalgae. EPA productivity was 70.2 mg/L/d and 6.5 mg/L/d, respectively.^{165,173}

For EPA production, *Phaeodactylum* was known as photoautotrophic EPA producer. Meiser *et al.*

cultivated *Phaeodactylum tricornutum* UTEX 640 under continuous light in batch culture.¹⁷⁴ Maximal EPA productivity 118 mg/L/d were obtained. *Nannochloropsis* sp. was cultivated in flat plate reactor under 1000 $\mu\text{mol photon/m}^2/\text{s}$ gave 127.9 mg/L/d EPA.⁸⁷

Until now, *Pavlova* and *Isochrysis* were found as two of photosynthetic microalgae produced DHA. However, productivity of DHA production by these microalgae was less than that by non-photosynthetic microorganisms (table 3). The highest DHA productivity was 4.3 mg/L/d, obtained when cultured *Isochrysis galbana* in optical fibre photobioreactor.¹⁶⁹

Table 5. LC-PUFA productivity of photoautotrophic production.

LC-PUFAs	Strains	LC-PUFA productivity (mg/L/d)
GLA	<i>Spirulina platensis</i> M2 ¹⁶⁸	26.4
	<i>Spirulina platensis</i> KCTC AG20590 ⁹⁷	0.4
ARA	<i>Parietochloris incisa</i> comb. nov ¹⁶⁵	70.2
	<i>Porphyridium cruentum</i> IAM R-3 ¹⁷³	6.5
EPA	<i>Porphyridium cruentum</i> IAM R-3 ¹⁷³	3.6
	<i>Monodus subterraneus</i> UTEX 151 ¹⁷⁵	56.0
	<i>Monodus subterraneus</i> UTEX 151 ⁷²	25.7
	<i>Monodus subterraneus</i> UTEX 151 ¹⁶⁸	58.9
	<i>Phaeodactylum tricornutum</i> UTEX 640 ⁷⁸	19.0
	<i>Phaeodactylum tricornutum</i> UTEX 640 ⁷⁹	25.1
	<i>Phaeodactylum tricornutum</i> UTEX 640 ¹⁷⁵	50.0
	<i>Phaeodactylum tricornutum</i> UTEX 640 ¹⁷⁶	13.0
	<i>Phaeodactylum tricornutum</i> UTEX 640 ¹⁷⁴	118.0
	<i>Phaeodactylum tricornutum</i> UTEX 640 ¹⁷⁷	47.8
	<i>Phaeodactylum tricornutum</i> TFX-1 ¹⁷⁸	6.0
	<i>Nannochloropsis</i> sp. ⁸⁵	32.0
	<i>Nannochloropsis</i> sp. ⁸⁷	127.9
	<i>Nannochloropsis</i> sp. PP983 ¹²⁵	1.2
	<i>Pavlova lutheri</i> SMBA 60 ¹⁷⁹	0.5
	<i>Pavlova lutheri</i> SMBA 60 ¹⁴⁷	1.3
	<i>Isochrysis galbana</i> Parke ¹⁸⁰	4.8
	<i>Isochrysis galbana</i> ¹⁸¹	15.3
	<i>Isochrysis galbana</i> ¹⁸²	7.2
DHA	<i>Pavlova lutheri</i> SMBA 60 ¹⁷⁹	0.2
	<i>Pavlova lutheri</i> SMBA 60 ¹⁴⁷	0.7
	<i>Isochrysis galbana</i> UTEX 2307 ¹⁶⁹	4.3
	<i>Isochrysis galbana</i> CCMP 1324 ¹⁶²	0.6

	<i>Isochrysis galbana</i> CCAP 927/1 ¹⁸³	0.2
	<i>Isochrysis galbana</i> ¹⁸²	3.1

7. YIELD CONVERSION OF LC-PUFA PRODUCTION

Conversion yield is calculated on the ratio of production to substrate. It permits to evaluate

economical productivity of production. Table 6 showed conversion yield of biomass and LC-PUFAs in some LC-PUFA producers.

Table 6. Biomass and LC-PUFAs conversion yield of microorganisms.

Microorganisms	Substrate (S)	Y _{X/S} (g. g ⁻¹)	LC-PUFAs	Y _{LC-PUFA/S} (mg. g ⁻¹)	Modes of nutrition
<i>Mucor circinelloides</i> CBS 203.28 ³⁶	Acetic acid	0.30	GLA	10.00	H
<i>Cryptocodinium cohnii</i> ATCC 30772 ¹²³	Acetic acid	0.12	DHA	30.00	H
<i>Mortierella isabellina</i> ATHUM 2935 ²⁹	Glucose	0.50	GLA	7.70	H
<i>Mortierella alpina</i> DSA-12 ¹²⁸	Glucose	0.44	ARA	95.40	H
<i>Schizochytrium</i> G13/2S ¹⁸⁴	Glucose	0.39	DHA	64.02	H
<i>Aurantiochytrium limacinum</i> mh0186 ⁶¹	Glucose	0.38	DHA	71.67	H
Thraustochytrid G13 ¹⁸⁵	Glucose	0.46	DHA	56.25	H
<i>Cryptocodinium cohnii</i> ATCC 30 556 ¹⁴²	Glucose	0.50	DHA	38.59	H
<i>Cryptocodinium cohnii</i> ATCC 30772 ¹⁰¹	Glucose	0.37	DHA	21.27	H
<i>Nannochloropsis</i> sp. ⁸³	Glucose	0.20	EPA	8.75	M
<i>Nitzschia laevis</i> UTEX 2047 ¹²	Glucose	0.42	EPA	10.46	M

X: biomass; Y: conversion yield

8. IMPROVEMENT FOR LC-PUFA PRODUCTION

Microalgae cultivation in the large volume increases the productivity of biomass and LC-

PUFAs. Nowadays, a lot of photobioreactors were invented for microalgae culture.

Cultivation of non-photosynthetic microorganisms was carried out in the closed and sterile systems with the sources of organic carbon. Because of heterotrophic culture, light was not necessary in this system. Source of carbon usually is one of factors influencing to production. Thus, fed-batch or continuous culture were often used to improve LC-PUFA production.

Conversely, light was necessary in cultivation of photosynthetic microorganisms.¹⁸⁶ Thus, bioreactors can be designed to obtain light effectively. By using a new type of enclosed photobioreactor in which light was efficiently distributed by light diffusing optical fibres, DHA from *Isochrysis* was obtained 4.3 mg/L/d, twofold greater than that obtained using flat glass bottles.¹⁶⁹ *Nannochloropsis* sp. was cultured in flat plate reactor with a narrow (1 – 2 cm) light path and rigorous stirring exposed to a high photon flux densities (1000-3000 $\mu\text{mol photons/m}^2/\text{s}$). Biomass and EPA yield were obtained 40.6 g/L and 2302 mg/L, respectively.⁸⁷

Culture in two stages of temperature is a strategy for improvement of LC-PUFA production: the first stage for biomass production and the second for LC-PUFA production. In the second stage, temperature was usually decreased to produce more LC-PUFAs.

In *Pythium irregulare* ATCC 10951, cells were initially grown at 25° C for 1, 2 or 3 days and then shifted to 12° C for 6, 8, 9 days. The best combination was 2 days at 25° C, followed by 6 days at 12° C, which gave 93.1 $\mu\text{g/ml}$ EPA.⁴⁸ *Mortierella alpina* ATCC 32222 was cultured for 8 days at 25° C gave a high biomass (52.4 g/L) and ARA yield (9.1 g/L). Then, the culture was incubated at 15° C. The maximal ARA content was obtained (11.1 g/L) in 11 days of fermentation.¹⁸⁷ An increase in cellular DHA content by 19.9% and productivity by 6.5% was observed when the temperature in the culture of *Cryptocodinium cohnii* ATCC 30556 was shifted from 25° C for 2 days to 15° C for 1 day compared with that maintained at 25° C for 3

days.¹⁴⁰ A shift of temperature from 30° C for 32 h to 20° C for 12 h in the culture of *Schizochytrium* sp. HX-308 resulted in an increase of DHA content which is present 6.05% in dry cell weight and 51.98% in total fatty acids.¹⁸⁸ In *Cryptocodinium cohnii* CCMP 316, n-dodecane was added in the culture as an oxygen vector. The DHA content in total fatty acids, the DHA content in biomass and DHA yield increased by 16, 39 and 22%, respectively, at 0.5% n-dodecane.⁶³

The increase of DHA content (15.7 to 17.8 mg/g biomass) was also found when *Isochrysis galbana* LB 2307 was shifted from 24° C to 17° C for 24 h.¹⁶⁹ In the culture of *Phaeodactylum tricornutum* 2038, cells were cultivated at 25° C and then shifted to 20, 15, 10° C. An increase of EPA content per dry mass was observed after 12 h, 24 h and 48 h at 10° C, 15° C and 20° C, respectively. The highest EPA yield was 6.6 mg/L when temperature was shifted from 25° C to 10° C for 12 h, which raised by 120% compared with the control.¹³⁷ After decantation, biomass of *Skeletonema costatum* was obtained and incubated at 15° C for 15 h, resulted in an increase in EPA content from 11 mg/g to 19 mg/g of dry weight.⁹⁵

9. CONCLUSION

The limitation of essential LC-PUFA sources originated from animals and plants has promoted the researches on other sources. Microorganisms were found as potential sources for LC-PUFA production because they could grow fast on culture media and contain high LC-PUFA content in their cells. Besides heterotrophically LC-PUFA producing microorganisms, many microalgae have discovered as LC-PUFA producers. Among these microalgae, some strains could produce LC-PUFAs in 2 or 3 modes of nutrition. Among nutritional modes, heterotrophy was found as a mode of high productivity production. However, mixotrophy was also potential for improvement of LC-PUFA productivity in photosynthetic microorganisms. Further researches need to focus on new microalgal strains to diversify LC-PUFA sources.

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