Effect of nitrogen stress and temperature on the yield of some fatty acids *Lyngbya* algae

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Abstract: Microalgae are an alternative source of food, animal feed, biofuel, fertilizer, cosmetics, nutraceuticals, and pharmaceutical products. Growth rates of the microalgae, biomass output, and nutritional value as measured by the generation of lipid and fatty acids all have an impact on the extraction of organic components from microalgae grown in various nutrient compositions. Investigation on the significance of temperature and nitrogen, both of which are essential for algae growth, has been conducted. This study attempts to emphasize the temperature and nitrogen level needed for locally isolated microalgae (*Lyngbya sp)*.and focuses on the benefits of nitrogen and the effect of temperature for increasing biomass productivity of *Lyngbya sp* for improved lipid and fatty The effect of acid quantities. Locally isolated microalgae showed variation in the degree of response high temperatures (40)° for unsaturated fatty acids was the opposite of saturated fatty acids, as the highest concentrations of unsaturated fatty acids were recorded at temperature (20)° for each of(Docosatetraenoic acid, Oleic acid, Octadecatrienoic acid) and completely opposite for saturated fatty acids, which was recorded at temperature (40)° for each of (Tetradecanoic acid ,Pentadecanoic According to the quality of the fatty acids produced inside it as a result of ,acid, Hexadecanoic acid) a change when adding nitrogen from the nutrient medium, the medium recorded an increase in the rates of all saturated fatty acids at a concentration of 20, while unsaturated fatty acids recorded a decrease when nitrogen was added. in natural hosts (control),that can be used to utilize lipid and fatty acids from microalgae for biofuel and pharmaceutical, food supplements purposes

Keywords: Microalgae *Lyngbya* , fatty acids, nitrogen, , Temperature, food supplement

Introduction

During the last few decades, the capacity to use microalgae has drawn significant attention for a variety of reasons. In fact, these microbes have received a lot of attention as a potential source of biofuels (1) and bioactive ingredients for cosmetic and cosmeceutical formulations and expensive medications (2). Due to their ability to hold onto water and to slowly release macro- and micro nutrients into the soils around them, microalgae seem to be appropriate as fertilizers (3).Microalgae have also been investigated because they have the potential to provide nutritious food and feed additives(4) . In fact, microalgal biomass can serve as a starting point for third-generation (5). which anticipate its bio refinery processes conversion into a variety of commercial goods and energy, reducing expenses and waste generation (6) . To present, several businesses have embraced this strategy and are able to cultivate microalgae at pilot size and manufacture multiple goods from the same biomass, primarily energy carriers, pigments,

from the terminal carbon, which was discovered in algae in higher proportions (13) .

It is generally known that PUFA intake has multiple positive effects on human health, including increased metabolic rates, control of blood pressure and glucose levels, and defense against a wide range of illnesses, including certain cancers(14).

Moreover, EPA and DHA help protect chronic inflammatory illnesses and may reduce the risks of obesity in both people and animals(15). Several key PUFAs, such as linoleic acid (LA-C18:26) and -linolenic acid (ALA-C18:33), which are precursors to AA and DHA, respectively, cannot or are only imperfectly synthesized by humans and other animals(16) . As a result, these molecules must be directly absorbed from outside sources (17). Fish, mollusks, and crustaceans from the ocean, animal products (meat, milk, eggs), fungus, microbes, and plant sources are some of these sources(18) . for instance, some plants and microalgae (19) . Figure 1 summarizes the primary microbiological sources that can provide extremely valued lipids for nutraceutical uses, and polyunsaturated fatty acids, for various biotechnological industries(7) . The use of microalgae-based technology has a number of benefits that overcome several limitations associated with the production of other species and/or natural sources(8). They don't need arable land, so they don't compete for space with crops and have growth rates that are faster than those seen in terrestrial plants (9).

There is a growing interest in nutrition-based preventative and treatment options as the prevalence of chronic diseases connected to diet rises, Dietary polyunsaturated fatty acids (PUFA) have the ability to control the progression of chronic illnesses(10) . Long-chain PUFA research has received a lot of attention recently, fatty acids (FAs) with two or more double bonds in their acyl chain are referred to as polyunsaturated fatty acids (PUFA) (11). According to the length of their carbon backbone, PUFAs are divided into two main groups: short-chain polyunsaturated fatty acids PUFAs), with 16 or 18 carbon atoms, and long-chain polyunsaturated fatty acids (LCPUFAs), with more than 18 carbons (12) . The Greek letter in the PUFAs' nomenclature indicates the first position of the double bond

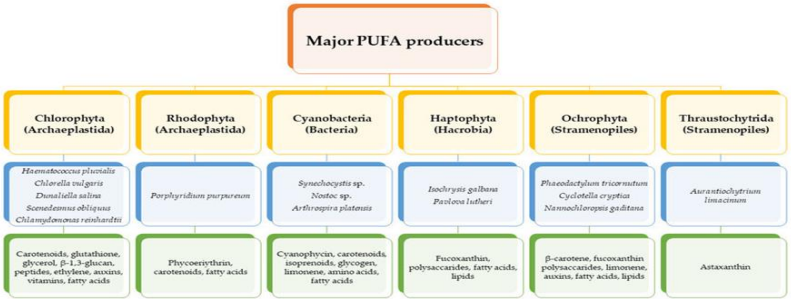

Figure 1 shows the primary microorganisms that produce polyunsaturated fatty acids (PUFAs) and the high-value products they are connected with(1)

actively bio synthesized during the (27) . While it often results exponential phase in a decrease in biomass and lipid yields, nitrogen (N) restriction or depletion is, to our knowledge, the most widely utilized culture alteration used to promote lipid synthesis (28). This issue can be effectively solved by two-stage cultivation systems, which consist of a nutrient-replenishment stage to increase biomass and a low- or non-N-supply stage to increase lipid production (29). These systems are also associated with a significant increase in the neutral lipid fraction, particularly triacylglycerols (TAGs) in the neutral lipid fraction, as an illustration, two-stage culture was discovered to be successful in raising lipid content in *Nannochloropsis spp.*(30).

Materials and methods

The specie of algae was isolated from the gharf river located in Thi-Qar .The studied algae is *Lyngbya* . Modified BG11was used for the algal growth Table 1 . Serial dilution method was used for algae isolation and purification in this study. For algae cultivation, 10 ml of isolated culture was added to a flask containing 100 ml of BG-11 and incubated for 14 days, then transported to 1000 ml of media and incubated for 14 days culture. The growth curve was determined for the studied alga.

Division : Cyanophyta

Class: Cyanophyceae

Order : Oscillatoriales

Family : Oscillatoriaceae

Genus : *Lyngbya sp*

Cell growth was measured by determining the optical density (O.P) daily. Optical density 650 nm) was measured by using

Among the factors that affect these fatty acids are as follows;

1- Temperature

Temperature can be used as a deciding factor for choosing the best strains to produce (20). In fact, PUFA-rich polar lipids temperature changes are very important in controlling membrane fluidity. In general lower temperatures increase the FAs' degrees of unsaturation, and vice versa (21).The productivity of all lipids, including PUFAs, can decrease with decreasing temperature, however, and the temperature circumstances that result in the highest PUFA production are often species-specific (22). In the oleaginous microalga *Scenedesmus obtusus* cultivated in outdoor plants, the impact of low (20 C) temperatures was assessed, in indoor cultures where higher temperature values were consistently maintained, a two-fold rise in PUFAs was see (23).At higher temperatures, the concentration of PUFAs often drops for instance, in the red alga *Porphirium purpureum*, EPA buildup likewise decreased significantly when the temperature rose (24) . In the diatom *Phaeodactylum tricornutum*, EPA, DHA, and other PUFA levels significantly decreased as temperature increased (from 15 to 25 C), (25)

2- Nitrogen

Lipid metabolism can be impacted by lipid metabolism in both the development phase and culture medium composition (e.g., nutrient content and composition), Unsaturated fatty acid (UFA) concentrations typically peak during the exponential phase and decline as cultures get older(26) . This occurs as a result of the higher quantities of UFAs found in plastidial membranes than in other cellular membranes and lipid droplets, which are

N: the optical density of the algal mass at the start of the experiment

$$G = \frac{0.301}{K}$$

spectrophotometer UV-VIS (540 nm .All measurements of the study were triplicates. The growth rate (K) and doubling time

$$K = \frac{\log N_t - \log N_0}{t}$$

.t: time

Nt: the optical density of the algal mass after .the passage of time t

Table 1: Components of BG11 culture medium

Salt	Stock solution	volume
Nano3	**150(g/L)**	**10**
K2HPO4	**4.0**	**10**
Mgso4.7H20	**7.5**	**10**
Cacl2.2H2o	**3.6**	**10**
Citric acid	**0.6**	**10**
Ferric citrate	**0.6**	**10**
EDTA-Na	**0.1**	**10**
Na2CO3	**2.0**	**10**
H3BO3	**61.0**	**1**
MnSo4.H2o	**169.0**	**1**
ZnSo4.7H2O	**287.0**	**1**
CuSo4.5H2O	**2.5**	**1**
NH4)6MO7O24.4H2	**12.5**	**1**

Experiment design:

harvested at the beginning of the stationary phase. Each culture of microalgae was centrifuged in the cooled centrifuge at 3000 rpm for 15 min, supernatant removed but organic precipitate had been washed with distilled water, and then dried at 45 Cº for two days. The dry weight was collected for extraction

Different concentrations of nitrogen was used in the current study, to stimulate the isolated algae for production lipid that can be used as biodiesel Dietary supplement and treatment. Nitrate was used as a source of nitrogen in media (NaNO3) and considered as control treatment in the study; .Microalgae had been

as an effective method for extracting fats from oily microorganisms such as yeast, fungi and microalgae)Samples were analyzed by GC mass system model.4.6 mm X 5µm injection flow 1 ml/min at UV.

RESULTS and Discussion

The growth curve was estimated in terms of optical density (650) Fig. (2) at (pH 7) and temperature (30)°C, and the exponential growth phase began after the seven day and reached the stable phase after the fourteenth day to begin the decreasing phase after the sixteenth day Its relative growth constant was (K = 0.0324) and the rate of doubling time (G = 13.35)

Lipid extraction and analysis

Ultrasound- assisted extraction (UAE).The UAE principle consists of forming sound waves in a liquid medium by exposure to ultrasonic waves (with a frequency of 20-50 kHZ) and creating high and low pressure cycles. During the high pressure cycle, the small vacuum bubbles formed in the low pressure cycle are destroyed intensively Which creates a phenomenon called cavitation. High pressure and jets formed during mechanical cavitation destroy the cellular structure of the reactant and intensify mass transfer processes, facilitating the extraction of fats.UAE technology is applied

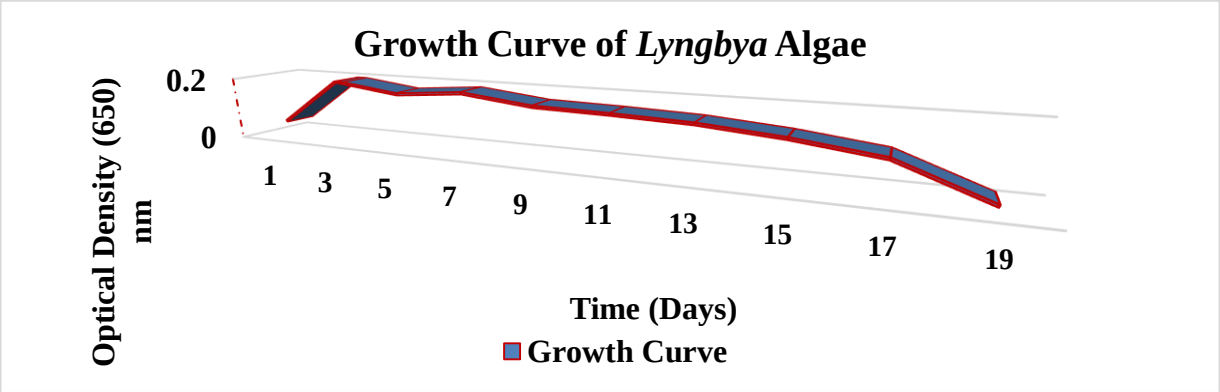

Figure 2;Growth Curve of *Lyngbya* Algae

Fatty acid content in the studied alga

Docosatetraenoic acid omega 6(8.07)% of total fat When normal growth control.

The effect of temperature on the level of fatty acids

Studies have shown that the chemical composition of algae in general and microalgae in particular is subject to change when growing in changing environmental conditions or when exposed to pollutants or the stage in which algae farms are harvested, also, algae vary in their biochemical content

The alga was investigated to study their lipids and essential omega fatty acids (3, 6, and 9) production capacity (*Lyngbya sp)* belonging to blue-green microalga

The content of *Lyngbya* alga varied in terms of unsaturated fatty acids (UFAs), as its content ranged from Octadecatrienoic acid, omega 6 (6.67)% and oleic acid (OA) (3.36%)and Eicosapentaenoic acid omega 3 (9.77)% and

Eicosapentaenoic acid omega 3 and Octadecatrienoic acid, omega 6, and oleic acid

and Similar with Qiao's study of the diatom *Phaeodactylum tricornutum*, an increase in temperature from 15 to 25 degrees Celsius led to a decrease in EPA, DHA and other PUFAs, counterproductive to saturated fatty acids.Temperature stress may induce changes in the FAs of cell membranes to avoid damage and be protected against the effects of increased temperature (34). This adaptation allows cyanobacteria and microalgae such as diatoms to survive in extreme conditions and involves remodeling membrane lipids by modifying FAs chain length and unsaturation to sustain the desired level of fluidity in cell .membranes (35)

Several authors have shown an inverse relationship between temperature and FAs .unsaturation in microalgae (36;37).

The results appeared for the saturated fatty acids, they were pentadecanoic acid (24.84)%, then tetradecanoic acid (5.94)% and hexadecanoic acid (2.07)%. When the temperature decreased, the percentages of the mentioned fatty acids decreased, and when the temperature increased, the percentages of these acids increased. As mentioned in Table 3

Content of unsaturated fatty acids under different temperatures of algae *Lyngbya* Table 2-

depending on the different algae species and nutrients available in the growth environment (31).

the temperature is one of the most significant , factors influencing how an alga is distributed in its surroundings. An unbalanced environment, as was observed to have an impact on the physical and chemical qualities both directly and indirectly in the environment where the algal cells reside, has an impact on both the biochemical content of those cells as well as their influence on many important critical processes (32)

The change in temperature showed a clear effect on the content of fatty acids in the alga. The highest content of Octadecatrienoic acid, omega 6, Docosatetraenoic acid omega 3 Eicosapentaenoic acid omega 3, and oleic acid was reached at the temperature (20) ° C (Table 2).

The results Similar to a study with Jethani who studied the effect of low temperatures (-20°C) on oily microalgae *Scenedesmus Obsetusus* is grown in outdoor plants. A twofold increase in PUFAs (33), also with Almeyda studied the effect of a decrease in temperature (from 20 to 11 °C) stimulating the production of PUFAs in *Cylindrotheca .closterium* during the stationary stage.

As for when the temperature (40) ° C was the Docosatetraenoic acid omega 3, then

isolates	temperature	RT	Area%	Compound name	Molecular formula	M.W
Lyngbya	20	24.107	12.2	**Octadecatrienoic acid , omega 6**	**C18H3402**	292
		24.106	6.39	**Oleic acid 9omega**	**C18H3402**	292
		14.958	10.07	**Eicosapentanoic acid omega 3**	**C20H30O2**	

		19.91	12.08	**Docosatetraenoic acid omega 6**	**C23H38O2**	
	30	24.107	6.67	**Octadecatrienoic acid , omega 6**	**C19H3202**	292
		24.106	3.39	**Oleic acid , omega9**	**C19H3202**	292
		21.853	9.77	**Eicosapentaenoic acid omega 3**	**C20H30O2**	
		19.91	8.07	**Docosatetraenoic acid omega 6**	**C23H38O2**	
	40	24.10	2.78	**Octadecatrienoic acid , omega 6**	**C19H3202**	292
		24.106	1.87	**Oleic acid , omega9**	**C18H3402**	292
		21.853	4.65	**Eicosapentanoic acid omega 3**	**C20H30O2**	346
		19.91	6.01	**Docosatetraenoic acid omega 6**	**C23H38O2**	

Table 3- Saturated fatty acid content under different temperatures of algae *Lyngbya*

Isolate	temperature	**RT**	**Area%**	**Compound name**	**Molecular formula**	
lyngbya	**20**	**15.88**	**17.07**	**Pentadecanoic acid-13 methyl-methyl ester(CAS)**	**C17H34O2**	**270**
		20.47	**3.94**	**Tetradecanoic acid**	**C14H28O2**	**366**
		21.65	**1.97**	**Hexadecanoic acid, 2(octadecyloxy)ethyl ester palmitic acid derivative**	**C36H72O3**	**552**
	30	**15.88**	**24.84**	**Pentadecanoic acid-14 methyl-methyl ester(CAS)**	**C17H34O2**	**270**
		28.08	**5.94**	**Tetradecanoic acid**	**C14H28O2**	**366**
		21.65	**2.07**	**Hexadecanoic acid, 2(octadecyloxy)ethyl ester-palmitic acid derivative**	**C36H72O3**	**552**
	40	**15.88**	**25.45**	**Pentadecanoic acid-14 methyl-methyl**	**C17H34O2**	**270**

				ester(CAS)		
		28.08	**3.21**	**Tetradecanoic acid**	**C14H28O2**	**366**
		21.65	5.34	**Hexadecanoic acid, 2(octadecyloxy)ethyl ester-palmitic acid derivative**	**C36H72O3**	**552**

The effect of nitrogen on the level of fatty acids

changes under the nutrient stress conditions, which is the indicator of photosynthesis and photochemical processes during which the energy accumulated in ATP is generated (41)

The current study is similar to that of Aljbory, who studied two locally isolated microalgae (*Chlorella vulgaris Bejerinck* and *Nitzschia palea*) to test their ability to produce

biodiesel by catalyzing different nitrogen concentration treatments (0, 2, 4, 8 g \ l), and the effect of nitrogen concentration on the amount of initial product

carbohydrates, protein), as well as the) quantity and quality of fat. Results revealed that nitrogen starvation led to elevated lipid production in *C. vulgaris* and *N. palea* Fat content increased from 6.6% to 40% and from 40% to 60% dry weight (DW),(42) And Montoya's study of the effects of temperature and nitrogen concentration on the lipid content of *Nannochloropsis oculata* and Chlorella vulgaris in view of their possible utilization as novel raw materials for biodiesel production

The lipid content of microalgae was strongly influenced by the variation of tested parameters; indeed, an increase in temperature from 20 to 25 °C practically doubled the lipid (FAS)content of *N. oculata* (from 7.90 to 14.92%), while an increase from 25 to 30 °C brought about a decrease of the lipid content

The most effective stimulus for inducing stress conditions in cells is nitrogen limitation, which increases lipid formation in microalgae (38).In a study on lipid content, Hu and colleagues found that, in nitrogen-limited environments, microalgae had lipid contents that range from 10 to 20% (39). According to the same study, cyanobacteria under stress produced less than 10% of their cells' total .lipid content

Nitrogen limitation is the most efficient stimulus for creating stress conditions in cells and therefore enhances lipid accumulation in microalgae (38). A lipid content study by Hu and colleagues showed that under conditions of nitrogen study reported that stress conditions in cyanobacteria caused less than 10% lipid content production in cells. Under nutrient stress conditions, microalgae will change the metabolism of fatty acids towards the synthesis and accumulation of triacylglycerol which consists of up to 80% of the total lipid content in the cell, The results are consistent with the current study (40). When nitrogen is limited in the cultivation medium, microalgae will accumulate large amounts of lipids but due to the lack of nitrogen the cells will not produce sufficient amounts of proteins which results in lower biomass production Chlorophyll content also

Docosatetraenoic acid (8.07)%, Oleic acid omega 9(4.39)%

When nitrogen increases, the percentages of these acids decrease to

Octadecatrienoic acid , omega 6(4.78)% , Docosatetraenoic acid (4.65)%, Eicosapentanoic acid omega 3 (2.54)% , Oleic acid omega 9(1.7)%

As for saturated fatty acids, it increases when nitrogen 20 is increased, as follows

Pentadecanoic acid(26.45)% , Tetradecanoic acid(8.21)%, Hexadecanoic acid(3.34)% It decreases when the nitrogen concentration decreases

Pentadecanoic acid(18.54)% , Tetradecanoic acid(2.5)%, Hexadecanoic acid(1.7)% As shown in the table 4,5 below;

of *C. vulgaris* from 14.71 to 5.90%. On the other hand, a 75% decrease of the nitrogen concentration in the medium, with respect to the optimal values for growth, increased the lipid fractions of *N. oculata* from 7.90 to 15.31% and of *C. vulgaris* from 5.90 to 16.41%, respectively.

And in another study by Wang, the effect of N reduction on lipid and fatty acid content Profiles in different lipid classes of *Phaeodactylum tricornutum, Isochrysis* af Microalgae cells were cultured in two different ways, batch and semi-continuous Culture, to create strong and medium constraints on N, which in turn will have a significant impact Biomass and fat productivity(43).

We notice that when nitrogen decreases, the percentage of unsaturated fatty acids increases as follows;

Eicosapentanoic acid omega 3 (11.07)% , Octadecatrienoic acid , omega 6 (8.67) %,

Table 4: Effect of nitrogen on unsaturated fatty acids

isolates	N	RT	Area%	Compound name	Molecular formula	M.W
Lyngbya	0	24.107	8.67	**Octadecatrienoic acid , omega 6**	**C18H3402**	292
		24.106	4.39	**Oleic acid omega 9**	**C18H3402**	292
		21.853	11.07	**Eicosapentanoic acid omega 3**	**C23H38O2**	346
		19.91	8.07	**Docosatetraenoic acid omega6**	**C23H38O2**	346
	5	24.107	7.07	**- Octadecatrienoic acid , omega 6**	**C18H3402**	292

		24.106	3.30	**Oleic acid omega 9**	**C18H3402**	292
		21.853	9.47	**Eicosapentanoic acid omega 3**	**C23H38O2**	346
		19.91	6.57	**Docosatetraenoic acid omega6**	**C23H38O2**	346
	10	24.107	6.67	**- Octadecatrienoic acid , omega 6**	**C19H3202**	292
		24.106	3.39	**Oleic acid , omega 9**	**C19H3202**	292
		19.91	3.08	**Docosatetraenoic acid omega 6**	**C23H38O2**	346
		21.853	9.77	**Eicosapentaenoic acid omega 3**	**C20H30O2**	
	20	19.58	4.78	**- Octadecatrienoic acid , omega 6**	**C19H3202**	292
		21.853	2.54	**Eicosapentaenoic acid omega 3**	**C20H30O2**	
		19.76	1.7	**Oleic acid , omega 9**	**C18H3402**	292
		19.91	4.65	**Docosatetraenoic acid omega 6**	**C23H38O2**	346

Table 5: Effect of nitrogen on saturated fatty acids

Isolate	**N**	**RT**	**Area%**	**Compound name**	**Molecular formula**	
lyngbya	**0**	**15.88**	**18.57**	**Pentadecanoic acid-13 methyl-methyl ester(CAS)**	**C17H34O2**	**270**
		20.47	**2.5**	**Tetradecanoic acid**	**C14H28O2**	**366**
		21.65	**1.7**	**Hexadecanoic acid, 2(octadecyloxy)ethyl ester palmitic acid**	**C36H72O3**	**552**

				derivative		
	5	15.88	20.5	Pentadecanoic acid-13 methyl-methyl ester(CAS)	C17H34O2	270
		20.47	5.01	Tetradecanoic acid	C14H28O2	366
		21.65	1.99	Hexadecanoic acid, 2(octadecyloxy)ethyl ester palmitic acid derivative	C36H72O3	552
	10	15.88	24.84	Pentadecanoic acid-14 methyl-methyl ester(CAS)	C17H34O2	270
		28.08	5.94	Tetradecanoic acid	C14H28O2	366
		21.65	2.07	Hexadecanoic acid, 2(octadecyloxy)ethyl ester-palmitic acid derivative	C36H72O3	552
	20	15.88	26.45	Pentadecanoic acid-14 methyl-methyl ester(CAS)	C17H34O2	270

Conclusions

Microalgae are a promising feedstock for many valuable biological products, including health-beneficial PUFAs that are mostly obtained from animal sources so far, as well as biofuels and others, and address several aspects to make microalgae production on an industrial scale viable, including This is the improvement of growth conditions and pre-treatment to increase the productivity of biomass and fats. As in a current study of algae *Lyngbya*, the extent to which the conditions affecting the production of fatty .acids are known

Reference

1- **Santin**, A., Russo, M. T., Ferrante, M. I., Balzano, S., Orefice, I., & Sardo, A. (2021). Highly valuable polyunsaturated fatty acids from microalgae: Strategies to improve their yields and their potential exploitation in aquaculture. *Molecules*, *26*(24), 7697.

2- **Elalami**, D., Oukarroum, A., & Barakat, A. (2021). Anaerobic digestion and agronomic applications of microalgae for its sustainable valorization. *RSC advances*, *11*(43), 26444-ealth? A systematic review of 26462. evidence. Crit. Rev. Food Sci. Nutr. 2019, 59, 1684–1727.

3- **Nieri**, P., Carpi, S., Esposito, R., Costantini, M., & Zupo, V. (2023). Bioactive Molecules from Marine Diatoms and Their Value for the Nutraceutical Industry. *Nutrients*, *15*(2), 464.

4- **Rumin,** J., Nicolau, E., Gonçalves de Oliveira Junior, R., Fuentes-Grünewald, C., & Picot, L. (2020). Analysis of scientific research driving microalgae market opportunities in Europe. *Marine drugs*, *18*(5), 264.

5- Benti, N. E., Gurmesa, G. S., Argaw, T., Aneseyee, A. B., Gunta, S., Kassahun, G. B., ... & Asfaw, A. A. (2021). The current status, challenges and prospects of using biomass

potential strategy against SARS-CoV-2 infection: a narrative review. Medicina, 57(12), 1389

15- **Zúñiga**-Hernández, J., Sambra, V., Echeverría, F., Videla, L. A., & Valenzuela, R. (2022). N-3 PUFAs and their specialized pro-resolving lipid mediators on airway inflammatory response: beneficial effects in the prevention and treatment of respiratory diseases. *Food & Function*, *13*(8), 4260-4272.

16- Telle-Hansen, V. H., Gaundal, L., Bastani, N., Rud, I., Byfuglien, M. G., Gjøvaag, T., ... & Myhrstad, M. C. (2022). Replacing saturated fatty acids with polyunsaturated fatty acids increases the abundance of Lachnospiraceae and is associated with reduced total cholesterol levels—a randomized controlled trial in healthy individuals. *Lipids in Health and Disease*, *21*(1), 1-13.

17- **Ramos-Lopez**, O., Martinez-Urbistondo, D., Vargas-Nuñez, J. A., & Martinez, J. A. (2022). The Role of Nutrition on Meta-inflammation: Insights and Potential Targets in Communicable and Chronic Disease

18- **Siddiki,** S. Y. A., Mofijur, M., Kumar, P. S., Ahmed, S. F., Inayat, A., Kusumo, F., ... & Mahlia, T. M. I. (2022). Microalgae biomass as a sustainable source for biofuel, biochemical and biobased value-added products: An integrated biorefinery concept. *Fuel*, *307*, 121782.

19- **Dineshbabu,** G., Goswami, G., Kumar, R., Sinha, A., & Das, D. (2019). Microalgae–nutritious, sustainable aqua-and animal feed source. *Journal of Functional Foods*, *62*, 103545.

20- **Banerjee**, S., Banerjee, S., Ghosh, A. K., & Das, D. (2020). Maneuvering the genetic and metabolic pathway for improving biofuel production in algae: Present status and future prospective. *Renewable and Sustainable Energy Reviews*, *133*, 110155.

21- **Shekh**, A., Sharma, A., Schenk, P. M., Kumar, G., & Mudliar, S. (2022). Microalgae cultivation: photobioreactors, CO2 utilization, and value-added products of industrial energy in Ethiopia. Biotechnology for Biofuels, 14(1), 1-24

6- **Gifuni,** I., Pollio, A., Safi, C., Marzocchella, A., & Olivieri, G. (2019). Current bottlenecks and challenges of the microalgal biorefinery. *Trends in biotechnology*, *37*(3), 242-252.

7- **Daly**, G., Ghini, V., Adessi, A., Fondi, M., Buchan, A., & Viti, C. (2022). Towards a mechanistic understanding of microalgae–bacteria interactions: integration of metabolomic analysis and computational models. *FEMS Microbiology Reviews*, *46*(5), fuac020.

8- **Ciani**, M., Lippolis, A., Fava, F., Rodolfi, L., Niccolai, A., & Tredici, M. R. (2021). Microbes: food for the future. *Foods*, *10*(5), 971.

9-Martínez, S. A. H., Melchor-Martínez, E. M., Hernández, J. A. R., Parra-Saldívar, R., & Iqbal, H. M. (2022). Magnetic nanomaterials assisted nanobiocatalysis systems and their applications in biofuels production. *Fuel*, *312*, 122927.

10- **Djuricic,** I., & Calder, P. C. (2021). Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: An update for 2021. *Nutrients*, *13*(7), 2421.

11- Abd- Al Sahib Issa ,M, 2013 Capability of production of unsaturated fatty acids (Omega-3 ,Omega-6) from local isolate of Mucor sp. and effect some growth factors for productivity increase .M.Sci,College of Science-University of Thi-Qar

12- **Story,** M. J. (2021). Essential sufficiency of zinc, ω-3 polyunsaturated fatty acids, vitamin D and magnesium for prevention and treatment of COVID-19, diabetes, cardiovascular diseases, lung diseases and cancer. *Biochimie*, *187*, 94-109.

13-**Al-Hussainawi**, Safaa, 2015. The biochemical content of some microalgae and the effect of some environmental factors and pollutants on unsaturated fatty acids. M.Sci, College of Science-University of Thi-Qar

14- Ferro, Y., Pujia, R., Maurotti, S., Boragina, G., Mirarchi, A., Gnagnarella, P., & Mazza, E. (2021). Mediterranean diet a

elevated CO2 concentrations on changes in fatty acids and amino acids of three species of microalgae. *Phycologia*, *59*(3), 208-217.

31- **Talebi,** S., Edalatpour, A., & Tavakoli, O. (2022). Algal biorefinery: a potential solution to the food–energy–water–environment nexus. *Sustainable Energy & Fuels*, *6*(11), 2623-2664.

32- **Sarwer**, A., Hamed, S. M., Osman, A. I., Jamil, F., Al-Muhtaseb, A. A. H., Alhajeri, N. S., & Rooney, D. W. (2022). Algal biomass valorization for biofuel production and carbon sequestration: a review. *Environmental Chemistry Letters*, *20*(5), 2797-2851.

33- **Abinandan,** S., Subashchandrabose, S. R., Venkateswarlu, K., & Megharaj, M. (2019). Soil microalgae and cyanobacteria: the biotechnological potential in the maintenance of soil fertility and health. *Critical reviews in biotechnology*, *39*(8), 981-998.

34- **Jethani,** H.; Patel, P.; Mudliar, S.N.; Sarada, R.; Chauhan, V.S. Growth and biochemical response of an indigenous oleaginous microalga Scenedesmus obtusus cultivated in outdoor open ponds. Indian J. Exp. Biol. 2019, 57, 40–49.

35- **de la Rosa** F, de Troch M, Malanga G, Hernando M (2020). Differential sensitivity of fatty acids and lipid damage in Microcystis aeruginosa (cyanobacteria) exposed to increased temperature. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 235: 108773

36- **Sinensky** M (1974). Homeoviscous adaptation—Homeostatic process that regulates viscosity of membrane lipids in Escherichia coli. Proceedings of the National Academy of Sciences of the United States of America 71: 522–525.

37- **Wada** H, Gombos Z, Murata N (1990). Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. Nature 347: 200–203.

38- **Zhu** CJ, Lee YK, Chao TM (1997). Effects of temperature and growth phase on lipid and biochemical composition of Ishochrysisgalbana TK1. Journal of Applied

importance. *Journal of Chemical Technology & Biotechnology*, *97*(5), 1064-1085.

22- Daneshvar, E., Wicker, R. J., Show, P. L., & Bhatnagar, A. (2022). Biologically-mediated carbon capture and utilization by microalgae towards sustainable CO2 biofixation and biomass valorization–A review. *Chemical Engineering Journal*, *427*, 130884.

23- **Dolganyuk**, V., Belova, D., Babich, O., Prosekov, A., Ivanova, S., Katserov, D., ... & Sukhikh, S. (2020). Microalgae: A promising source of valuable bioproducts. *Biomolecules*, *10*(8), 1153.

24- Ren, X., Liu, Y., Fan, C., Hong, H., Wu, W., Zhang, W., & Wang, Y. (2022). Production, Processing, and Protection of Microalgal n-3 PUFA-Rich Oil. *Foods*, *11*(9), 1215.

25-**Andrade**, D. S., Amaral, H. F., Gavilanes, F. Z., Morioka, L. R. I., Nassar, J. M., de Melo, J. M., ... & Telles, T. S. (2021). Microalgae: cultivation, biotechnological, environmental, and agricultural applications. *Advances in the domain of environmental biotechnology: microbiological developments in industries, wastewater treatment and agriculture*, 635-701.

26- **Alazaiza,** M. Y., Albahnasawi, A., Al Maskari, T., Abujazar, M. S. S., Bashir, M. J., Nassani, D. E., & Abu Amr, S. S. (2023). Biofuel Production Using Cultivated Algae: Technologies, Economics, and Its Environmental Impacts. *Energies*, *16*(3), 1316.

28-**Trovão**, M., Schüler, L. M., Machado, A., Bombo, G., Navalho, S., Barros, A., ... & Varela, J. (2022). Random mutagenesis as a promising tool for microalgal strain improvement towards industrial production. *Marine drugs*, *20*(7), 440.

29- **Chandra,** R., Iqbal, H. M., Vishal, G., Lee, H. S., & Nagra, S. (2019). Algal biorefinery: a sustainable approach to valorize algal-based biomass towards multiple product recovery. *Bioresource technology*, *278*, 346-359.

30- **Liang,** C., Wang, L., Zhang, Y., Zhang, J., Zhang, X., & Ye, N. (2020). The effects of

and lipid production of microalgae for bioenergy application. Online J Biol Sci 15:260

43- **Hassan**, F. M., Aljbory, I. F., & Kassim, T. I. (2013). An attempt to stimulate lipids for biodiesel production from locally isolated microalgae in Iraq. Baghdad Science Journal, .10(1), 97-108

44-Wang, X., Fosse, H. K., Li, K., Chauton, M. S., Vadstein, O., & Reitan, K. I. (2019). Influence of nitrogen limitation on lipid accumulation and EPA and DHA content in four marine microalgae for possible use in aquafeed. *Frontiers in Marine Science*, *6*, 95

Phycology 9: 451–457. DOI 10.1023/ A:1007973319348.

39- **Sulochana** SB, Arumugam M (2020) Targeted metabolomic and biochemical changes during nitrogen stress mediated lipid accumulation in Scenedesmus quadricauda CASA CC202. Front Bioeng

40- **Hu** Q, Sommerfeld M, Jarvis E et al (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639

41- **Ratomski** P, Hawrot-Paw M (2021) Influence of nutrient-stress conditions on Chlorella vulgaris biomass production and lipid content. Catalysts 11:573

42- **Rai** MP, Gautom T, Sharma N (2015) Effect of salinity, pH, light intensity on growth