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ENHANCING ACCUMULATION OF OMEGA 3 AND 9 FATTY ACIDS IN *CHLORELLA VULGARIS* UNDER MIXOTROPHIC NUTRITION

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ABSTRACT

Omega fatty acids have critical part in enhancing general health and protection against serious diseases. The present work showed the distinctive nutritional regimes on *Chlorella vulgaris* which resulted in switch of metabolic pathways that not only prompted the dry weight and lipid yield, but also upgraded the yield of special fatty acids, omega 3 and 9 fatty acids, which consequently improved the food quality for human and aqua-creatures. High biomass and lipid production were accomplished by either cultivating *C. vulgaris* at low nitrogen content medium and/or mixotrophic nutrition by glycerol or sugarcane molasses. Glycerol supplementation promoted the dry weight achieving 171% at 0.1 M and 185% at 0.2 M, while the highest total lipid yield (179%) recorded at 0.1 M. GC–MS qualitative and quantitative analysis showed the high yield of α-Linolenic acid to 281%, Eicosapentaenoic acid to 207% at glycerol (0.1 M). Elaidic acid (C18:1t ω9) enhanced almost 14 times, while Erucic acid (C22:1 ω9) enhanced to 256% under glycerol supplementation to growth medium.

Key words: *Chlorella vulgaris*, Omega 3, 9, mixotroph, fatty acids, gas chromatography.

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INTRODUCTION

Omega-3, omega-6 and omega-9 fatty acids are considered fundamental fats for healthy body. These fatty acids differ in chain length, number and position of double bonds and counted from the methyl end (Ruiz-López, *et al.*, 2012). Amongst omega 3 unsaturated fats, Eicosapentaenoic acid (EPA, C20:5) and Docohexaenoic acid (DHA, C22:6) are considered the most essential polyunsaturated fatty acids (PUFAs). On the other hand, α-Linoleic acid (ALA, C18:1) and Arachidonic acid (AA, C20:4) have several medical advantages (Kapoor, *et al.,* 2011; Lane and Derbyshire, 2017). Omega-9 fatty acids contain just a single double bond, so they are known as monounsaturated fats. Oleic acid is a standout amongst the most important omega-9 fatty acids that can be synthesized by the human body. Therefore, omega-9 fatty acids are viewed as the most copious fats in many cells in the body and repress TRPV1 channels that prompt alleviation in pain and itch (Morales-Làzaro *et al.,* 2016). Contrasted with omega 6 and omega 9, omega 3 fatty acids are found to have an essential part in enhancing heart wellbeing by means of decreasing triglycerides level (Rizos and Elisaf, 2017), cancer treatment (MacLean *et al.,* 2006), supporting the psychological well-being through diminishment of manifestations of schizophrenia and dejection (Freemantle *et al.,* 2006), supporting mind and visual capacity (Nguyen *et al.,* 2014) and recovering inflammation (Li *et al*., 2014). Traditional investigations reveal that aquacultures are found to be the fastest and the best recommended source for omega 3 fatty acids (Klinger and Naylor, 2012). Currently, microalgae act as essential food supply to aquatic organisms for their role in balance the nutritional composition as well as production bioactive compounds (Schafberg *et al.,* 2018). Since microalgae can accumulate high omega 3 concentrations, aquatic organisms gain these fatty acids through the food chain. For this reason, microalgae become potential source for omega 3 fatty acids due to their high productivity per unit area, use of non-arable land and hard to be contaminate (Chisti, 2007; Ryckebosch *et al.,* 2014). Numerous marine microalgal and macroalgal species can produce various kinds of unsaturated fats, including omega 3, 6 and 9 fatty acids (Lim *et al*., 2012). In addition, a few marine and freshwater microalgae can accumulate total lipid achieving 80% of their dry weight (Burja *et al.,* 2006). Switching nutritional behaviour toward mixotrophic regime promoted induction the growth rate, lipid content and consequently the lipid yield (Chen and Walker, 2011; Liu *et al.,* 2013). Few conditions may control the accumulation of omega 3 fatty acids in microalgae including growth stages, kind of nutrition and/or stress conditions (Cossins *et al.,* 2002). Additionally, fatty acids analysis of some microalgae revels that the proportions of omega 3 to omega 6 fatty acids are strain subordinate. One of these strains is *Chlorella vulgaris* that can gather high lipid content

including omega 3 and 6 unsaturated fats (Abdo *et al.,* 2015). Monitoring the metabolic pathways and genetic engineering are considered effective routs to upgrade omega 3 fatty acids production (Hamilton *et al.,* 2014). The simplicity of *C. vulgaris* cell structure paves the way for to enhance the production of omega-3 and 9 via controlling the cultivation condition. For this reason, the present work shows a basic, quick and appropriate strategy for high accumulation of omega 3 and 9 fatty acids by means of changing the nutrition regime of *Chlorella vulgaris*.

MATERIALS AND METHODS

Cultivation conditions: *Chlorella vulgaris* was cultivated in Kessler and Cyagan medium (KC medium) as described by Kessler and Cyagan (1970). A *Chlorella vulgaris* inoculum (400 mL) was loaded in 1 liter capacity Erlenmeyer jars and shacked at 25 ±1°C for 24 hours under white light fluorescent enlightenment (80 µmol/photons/m/s). To achieve optimum pH value, a wide pH range was performed using phosphate buffer (Sambrook and Russell, 2001) and borax buffer (Robinson and Stokes, 1968). For Nitrogen stress, different potassium nitrate (KNO_3) concentrations took separately, (0% 0 mM), 25% (2 mM), 50% (4 mM), 75% (6 mM), 100% (8 mM) and 150% (12 mM). Concerning carbon enrichment nutrition, glycerol supplied reaching final concentration of [0 M (control), 0.05 M, 0.1 M and 0.2 M] as following [0 g/L, 4.60 g/L, 9.21 g/L, 18.42 g/L], while sugarcane molasses supplied in (0, 1, 3 and 5 g/L). The pH-values adjusted after inducing either glycerol or molasses. After optimization of each parameter, the KC medium at pH (7) marked as control, KC medium containing low nitrogen content [2 mM KNO_3 (25%)] labelled as nitrogen deficiency, 0.1 M glycerol in KC medium labelled as mixotrophic glycerol, 3% sugarcane molasses in KC medium labelled as mixotrophic molasses, and KC medium containing 2 mM KNO_3, 1 mM glycerol, 3% sugarcane molasses labelled as mixed condition.

Growth estimation (mg/mL): Algal cultures were monitored by optical density, where cells assembled at OD_{750nm} = 2.5±0.5. After 14±2 days, samples of 30 mL of algal culture centrifuged at 5000 g for 10 min and washed three times with distilled water and then moved into a pre-weighted petri-dish and dried in an oven at 60 ºC until a constant weight gained. Growth expressed as cell dry weight (CDW).

Lipid yield Estimation (mg/L): Algal lipid yield estimated according to Chu and Ozkizilcik (1995). The weight of total lipid production (lipid yield) in 30 mL divided by 0.03 and expressed as (mg/L) of algal culture.

Lipid content (mg Lipid /g Dry weight): Lipid content was figured by isolating the produced lipid yield extracted from 30 mL of an algal culture by cell dry weight from 30 mL of an algal culture. Lipid content expressed as mg lipid/g dry weight.

Fatty acids analysis: Extracted lipids were performed to fatty acid methyl ester by dissolving in 4 mL benzene and 20 mL of 1% sulfuric acid in absolute methanol (Radwan, 1978). Extracted FAMEs were identified and quantified using GC-MS (7890A GC system, USA).

Chromatographic conditions: Carrier gas, helium, flow rate, 1.5 ml/min, sample input temperature, 290 °C initial temperature, 90 °C for 1 min, detector temperature, 300 °C, capillary column, HP-5MS, length 30 m, diameter 0.25 mm were selected. FAs were identified by mass spectra and compared for retention times with those of standards ("Sigma", USA).

Statistical analysis: Mean of five recoded data appeared with ± standard Error (SR). The statistical analysis was computed by IBM SPSS (Statistics 20).

RESULTS AND DISCUSSION

Influence of hydrogen ion concentration on lipid yield: *Chlorella vulgaris* exhibited high growth rate at neutral pH value, where it showed the highest recorded dry weight (4.79 mg/mL). A significant decrease in dry weight was observed by either raising or bringing down the hydrogen ion concentration (Table 1). Likewise, cell lipid content was influenced by hydrogen ion concentration. The 17% and 28% inhibition in cell lipid content observed due to bringing and raising pH value to 5.5 or 9, respectively. The most elevated lipid yield acquired at pH 7 (Table 1). These results came in agreement with that reported by Violeta *et al.,* (2011) who showed that the highest growth rate of *Chlorella* sp. obtained in the range of 6.0 to 9.0, yet began to decrease from pH 5 and the highest triglyceride and lipid yield of *Chlorella vulgaris* recorded at pH 7 (Violeta *et al.,* 2011).

These upgrades may relate to advancement of supplements nutrients uptake, cell activity and/or enhancement of photosynthetic rate (James *et al.,* 2013).

Influence of nitrogen starvation on lipid yield: Increase KNO_3 concentration within media to 4 mM brought about 20% improvement of *Chlorella vulgaris* dry weight which relatively kept almost steady at 8 and 12 mM. Conversely, cell lipid content showed an inverse relationship at low nitrogen concentration. Cell lipid content gradually decreased to 142 mg/g at 12 mM KNO_3. Lipid yield showed the highest value at KNO_3 concentration of 4 mM, 1.156 mg/mL, although the highest cell lipid content showed a negative relationship to nitrogen concentration (Table 1). *Chlorella vulgaris* is

able to accumulate lipid up to 40% under low nitrogen-containing media (Illman*et al.,* 2000). The low dry weight of nitrogen may due to decrease in photosynthetic pigments and photosynthesis (Gordillo*et al.,* 1998). Also, lipid may accumulate under N-deficient because binding of available nitrogen to fundamental cell structures and enzymes, so fixed carbons are converted into sugar or lipid rather than to protein (Richardson *et al.,* 1969; Yeesang and Cheirsilp, 2011).

Lipid production under mixotrophic regimes: Glycerol included most anabolic and catabolic pathways within algal cell, so it showed advancing impact on the growth of *Chlorella vulgaris*. The increase in 14% dry weight was recorded in the culture media supplemented by 0.05 M glycerol. By raising glycerol concentration from 0.1 M to 0.2 M, algal dry weight promoted to 171% and 186%, respectively. Low glycerol concentration, 0.05 M, promoted lipid content to 136% as compared to control, while higher glycerol concentrations exhibited a negative response toward lipid content. So, the highest content lipid yield (178%) observed at 0.1 M glycerol (Table 2). Acquired outcomes showed that sugarcane molasses improved *Chlorella vulgaris* dry weight. The most elevated dry weight, 134% recorded at 1 g/l molasses concentration. Cell lipid content and total lipid yield displayed nearly a similar behavior, where they exhibited the most astounding advancing percent at 3 g/l molasses, 182 and 200%, for cell lipid content and total lipid yield, respectively (Table 2). Hence, 3 g/l sugarcane molasses was chosen for further omega 3, 6 and 9 investigations. Lipid yield expanded to 178% and 200% (Table 2) under mixotrophic nutrition by glycerol and sugarcane, respectively. Obtained results were supported by several literatures, who affirmed the improvement of algal biomass and lipid content in response to mixotrophic nutrition by sugarcane molasses (Liang *et al*., 2009; Kong *et al*., 2013; Hee-Jeong and Sung-Whan, 2015). EL-Sheekh*et al.* (2014) showed that cell lipid yield of *Chlorella vulgaris* and *Scenedesmus obliquus* improved by supplementing molasses in culture medium.

Fatty acids profiles under different nutritional regimes:

- *Yield of omega 3, omega 6 and omega 9.*

Under mixotrophic nutrition with 0.1 M glycerol, α-Linolenic acid (C18:3α ω3) enhanced up to 281% contrasted with control, while supplementation with 3% sugarcane molasses did not show critical changes (Table 3). Other treatments restrained the creation of α-Linolenic acid (C18:3α, ω3). Concerning Eicosapentaenoic acid (C20:5 ω3), it improved by mixotrophic nutrition to 207% and 179% for 0.1 M glycerol and 3% molasses, respectively. Eicosatrienoic acid (C20:3ω3) was observed steady in all treatments (Table 3). Lessening of γ-Linolenic acid (C18:3γ ω6) observed due to mixotrophic nutrition or nitrogen stress, where cultivation the algal cells on nitrogen insufficiency condition (2 mM KNO_3) prompted γ-Linolenic acid by 11% contrasted with control. Despite the fact that Arachidonic acid (C20:4 ω6) had not been recorded in control medium, its level showed low variations among all treatment that ranged from 37.6 to 56 ppm. Eicosatrienoic acid (C20:3 ω6) was not influenced by neither nitrogen limitation nor mixotrophic nutrition, where it displayed almost steady quantities that ranged from 37 to 39 ppm. Mixotrophic nutrition by glycerol displayed high Oleic acid (C18:1c ω9) yield that reached 526%, while other treatments showed a decline of Oleic acid yield compared to control. Elaidic acid (C18:1t ω9) was also influenced remarkably by mixotrophic nutrition, either by glycerol or molasses, where almost 14 and 10 times upgraded of Elaidic acid observed due to mixotrophic nutrition by glycerol and molasses, respectively. Likewise, Erucic acid (C22:1 ω9) was elevated due to mixotrophic nutrition, where under glycerol and molasses nutrition, 256% and 140% were recorded contrasted with control, respectively.

The fatty acids profiles of alga cultivated on 2 mM KNO_3, 0.1 M glycerol, 3% molasses or a combination of all these conditions compared to that of control. The fatty acids profiles indicated high differences of omega 3, 6 and 9 fatty acids content due to low nitrogen supplementation and/or mixotrophic nutrition.

Despite cultivation under low nitrogen, 2 mM KNO_3 led to upgrade the production of Palmitic acid (154%), while omega 3, 6 and 9 fatty acids decreased to 27.8%, 28.8% and 65.9%, respectively, contrasted with control. Although, cell lipid content enhanced with nitrogen deficiency while dry weight showed a negative relationship. Present results came in agreement with that reported by Zhang *et al*. (2013) and Hui *et al*.(2017), who concluded that in spite of promising role of nitrogen deficiency on lipid yield, fatty acids analysis showed low omega 3, 6, 9 fatty acids. For this reason, cultivation of *Chlorella vulgaris* under nitrogen limiting conditions could be useful for biodiesel production but not for omega 3, 6, 9 fatty acids production.

Mixotrophic nutrition with 3 g/l molasses prompted induction of Palmitic acid by 15%, omega 3 fatty acids by 4%, omega 9 fatty acids by 27%, while omega 6 fatty acids reduced to 40% contrasted with control. Conversely, by adding 0.1 M glycerol to *C. vulgaris* cultures resulted in energizing outcome, where Palmitic acid content expanded to 301.9% and omega 3 fatty acids achieved 267% as compared to control. Despite glycerol supplementation upgraded monounsaturated omega 9 fatty acids to 570%, omega 6 fatty acids content reduced to 40% contrasted with control (Table 3). Beside reduction of total fatty acid content, algal cells grew on combined media; showed high reduction of omega 3, 6 and 9 fatty acids content

that achieved 28, 35 and 51%, respectively. Observed data showed wide variations of fatty acids content and composition in response to nutritional regimes. Despite, low nitrogen supplementation enhanced the production of saturated fatty acids by 48.5%, monounsaturated and polyunsaturated fatty acids reduced by 21.3% and 83.4%, respectively (Figure 1). Fatty acid profiles showed wide variations among different nutrition conditions, where the concentration of total recoded saturated fatty acids were 540, 4951, 6347, 2263 and 660 ppm for control, under nitrogen limitation, 0.1 M glycerol, 3% molasses and mixture cultivation conditions, respectively. The concentration of monounsaturated fatty acids showed close quantities, 817, 643 and 647 ppm, in case of control, nitrogen deficiency and mixture conditions, respectively. Supplementing of *Chlorella vulgaris* by 0.1 M glycerol and 3% molasses improved enlistment of monounsaturated fatty acids up to 3898 and 1072 ppm, respectively. All nutrition administrations showed a decrease in polyunsaturated fatty acids. This declining trend was high in nitrogen insufficiency and supplementation by 3% molasses, where 83% and 64% diminishment observed due to these conditions, respectively (Figure 1).

Obtained results support our hypothesis "algal fatty acids composition is very sensitive to abiotic conditions. These variations include chain length and/or existing and position of double bond(s)". Promoting the algal growth and lipid production from algae under mixotrophic nutrition were confirmed by several previous works (Colin 2004; Heredia-Arroyo *et al.,* 2010; Hee-Jeong and Sung-Whan, 2015). Glycerol was found to be an optimum alternative carbon source for high omega 3 and omega 9 fatty acids production. Hee-Jeong and Sung-Whan (2015) suggested that the anabolism in mixotrophic cultures might be accelerated due to high formation of adenosine triphosphate in photochemical reactions as well as heterotrophic reactions. Additionally, glycerol enhanced the production of Palmitic acids, which considered the precursor of other longer fatty acids including alpha-linolenic acids, Eicosapentaenoic acids, Oleic acid, Elaidic acid and Erucic acid (Colin, 2004).

Effect of different nutritional regimes on fatty acids chain length: Chains lengths of produced fatty acids were also influenced by nutritional regimes (Figure 2). Short chains fatty acids (6-15 carbon molecules) showed minor variations in case of control (7.6%), 0.1 M glycerol (6%), 3% molasses (6.5%) and mixture (8.7%) contrasted with total fatty acids content of the same treatment, whereas cells grown on low nitrogen concentration medium produced 39.8% of 6-15 carbon atoms fatty acids compared to total fatty acids.

Concerning C16-C17 chains fatty acids, they displayed relatively constant values throughout all treatments, where they ranged from 42.7% of nitrogen stress condition to 58.3% of mixture condition. Here, it should be emphasized that cells grew on low nitrogen condition (2 mM KNO_3); produced 82.5% C6-C17 carbon atoms fatty acids. Recorded fatty acids with 18 carbon atoms exhibited remarkable variations due to different nutritional conditions. Estimated C-18 carbon atoms fatty acids were 35.5% under control condition, 8% in case of low nitrogen supplementation, 41% in case of 0.1 M glycerol supplementation, 29.4% in case of supplementation by 3% molasses and 17.7% at mixture condition. Although C18 carbon atoms content was found to be the largest content in case of cultivating in 0.1 M glycerol, the same condition exhibited the lowest C20-24 fatty acid content, where 13%, 9.2%, 6.3%, 14.7% and 15.3% recorded for control, 2 mM KNO_3, 0.1 glycerol, 3% molasses and mixture cultivation conditions, respectively, compared to total fatty acid content of the same treatment (Figure 2). Short fatty acids under low nitrogen concentration may be resulted from conversion of several amino acids into organic acids (Hui *et al*., 2017), which consequently led to low protein content including enzymes (Wienkoop *et al*., 2010). So, it could be suggested that low nitrogen supplementation might disturb the lipid metabolism enzymes which consequently resulted in accumulation of low molecular weight fatty acids (Table 3 and Figure 2). Under low nitrogen concentration, high soaked unsaturated fatty acids upgraded nine-time compared to control, while monounsaturated unsaturated fatty acids, omega 9, were detected in high amount in case of glycerol supplementation.

Despite polyunsaturated fatty acids were relatively steady in *C. vulgaris* cells of control, glycerol containing media and mixture conditions, supplementation glycerol to algal medium enhanced omega 3 and reduced omega 6 contents compared to control. These results revealed that distinctive nutrition designs showed directly effect on metabolic pathways of lipids inside the algal cell, chain lengths and saturation ratio of fatty acids (Figures 1 and 2). Glycerol supplementation encouraged the metabolic pathway toward production of linolenic acid, oleic acid, and Elaidic acid. Interestingly, short chain fatty acids are extremely restricted in all treatments except for that of nitrogen stress that achieved 82.5% of total fatty acids content which may owe to cell protection system and/or enzyme content of the cell.

Table 1. Effect of different pH values and nitrogen concentration on dry weight, lipid content and lipid yield of *Chlorella vulgaris*.

pH stress				Nitrogen stress			
pH value	Growth mg/ml	Lipid content mg/g	Lipid yield mg/ml	KNO_3 (mM)	Dry weight mg/ml	Lipid content mg/g	Lipid yield mg/ml
5.5	3.50 ±0.1	153.33 ±3.07	0.537 ±0.023	**2**	3.94 ±0.076	238 ±2.1	0.938 ±0.015
6	4.02 ±0.04	168.6 ±6.08	0.678 ±0.046	**4**	5.75 ±0.093	201 ±1.5	1.156 ±0.014
7	4.79 ±0.16	185.37 ±6.07	0.874 ±0.024	**6**	5.67 ±0.085	169 ±1.9	0.958 ±0.016
8	4.31 ±0.14	113.51 ±4.08	0.489 ±0.032	**8**	4.66 ±0.072	148 ±1.5	0.690 ±0.011
9	3.51 ±0.13	132.8 ±3.67	0.466 ±0.024	**12**	4.28 ±0.088	142 ±1.8	0.608 ±0.016

Data are statically analysed using ONE-WAY ANOVA.

Table 2. Variation (%) of dry weight, lipid content and lipid yield of *Chlorella vulgaris* in response to mixotrophic nutrition by different concentrations of sugarcane molasses and glycerol.

	Control	Sugarcane Molasses (%)			Glycerol (M)		
Concentration	**0 %**	**1**	**3**	**5**	**0.05**	**0.1**	**0.2**
Dry weight (%)	100±5.2	134±6.5	110±4.8	105±5.5	114±6.3	171±5.9	186±5.7
Lipid content (%)	100±3.7	120±4.3	182±3.6	95±4.1	136±2.9	104±3.1	61.6±3
Lipid yield (%)	100±4.1	161±4.6	200±3.8	100±4.2	155±4.2	178±4.3	115±36

Data are statically analysed using ONE-WAY ANOVA.

Table 3. Concentration of Palmitic, omega 3, omega 6 and omega 9 fatty acids (ppm) in response to different nutritional conditions. Control: *Chlorella vulgaris* was cultivated in KC medium at pH 7, KC medium at 25% Nitrogen media containing 2 mM KNO_3, Glycerol: *Chlorella vulgaris* was cultivated in KC medium containing 0.1 M glycerol, Molasses: *Chlorella vulgaris* was cultivated in KC medium containing 3 g/l sugarcane molasses, Mixture: *Chlorella vulgaris* was cultivated in KC medium containing 2 mM KNO_3, 0.1 M glycerol, and 3 g/l sugarcane molasses.

	Fatty acid	Carbon atoms	Control	Nitrogen Limit.	Glycerol	Molasses	Mixture
	Palmitic acid	C16:0	1508.83	2323.77	4555.8	1732.52	1583.19
Omega 3	Linolenic acid methyl	C18:3α ω3	566.39	106.546	1593.592	559.284	87.45
	Eicosapentaenoic acid methyl	C20:5 ω3	41.536	37.554	85.976	74.228	58.39
	Eicosatrienoic acid methyl	C20:3ω3	33.814	34.188	34.958	33.484	33.44
	Total Omega 3 fatty acids	ω3	641.74	178.29	1714.53	666.996	179.28
Omega 6	γ-Linolenic acid methyl	C18:3γ ω6	369.34	41.646	150.062	89.276	49.324
	Arachidonic acid methyl	C20:4 ω6	0	37.598	37.356	37.642	56.23
	Eicosatrienoic acid methyl	C20:3 ω6	39.182	38.412	39.182	37.972	36.81
	Total Omega 6 fatty acids	ω6	408.52	117.66	226.6	164.89	142.36
Omega 9	Oleic acid methyl	C18:1c ω9	400.58	241.758	2108.26	211.354	202.6
	Elaidic acid methyl	C18:1t ω9	34.562	38.72	478.742	338.976	36.78
	Erucic acid methyl	C22:1 ω9	33.374	28.402	85.558	46.596	0
	Total Omega 9 fatty acids	ω9	468.51	308.88	2672.56	596.93	239.4

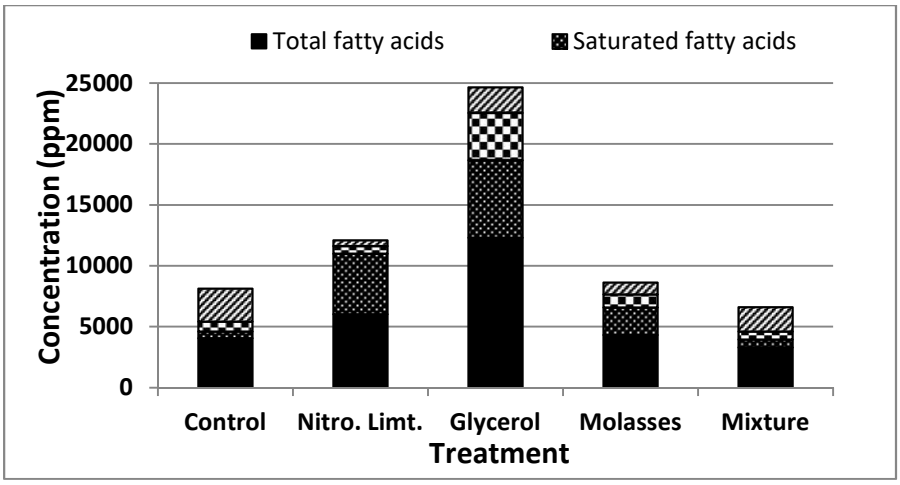

Figure 1. Concentration of total fatty acid contents, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids of Chlorella vulgaris under control, 25 % nitrogen supplementation, 0.1 M glycerol, 3% sugarcane molasses and a mixture of all treatment

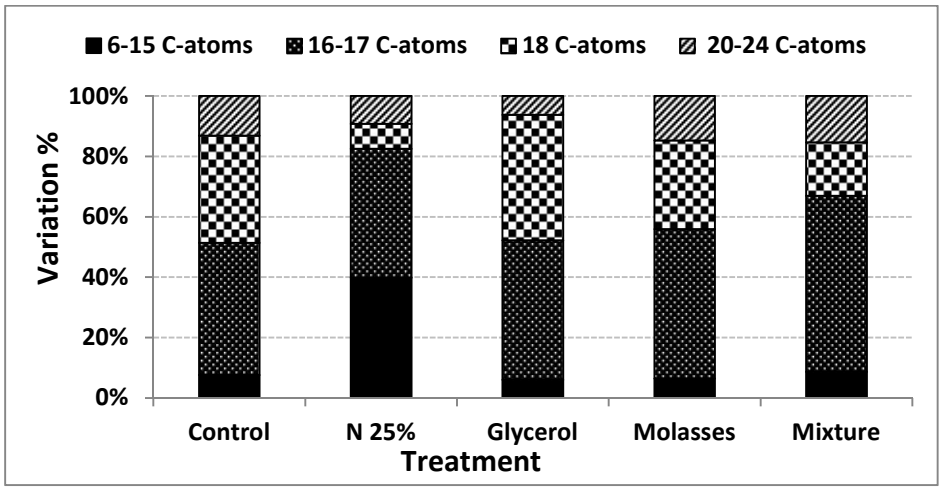

Figure 2. Variation of fatty acids carbon atoms content (%) in response to cultivation conditions. *Chlorella vulgaris* were grown in KC medium (control), 25 % nitrogen supplementation, 0.1 M glycerol, 3 % Sugarcane molasses and a mixture of all treatment.

Conclusions: Supplementation of *C. vulgaris* with molasses (3%) or glycerol (0.1 M) showed the highest total lipid yield. Moreover, the highest estimated omega 3 and omega 9 fatty acids recorded at glycerol (0.1 M), where α-Linolenic acids and Eicosapentaenoic acid enhanced to 281 and 207%, respectively. Regarding omega 9 fatty acids, Oleic acid reached 526 %, Elaidic acid promoted 14 time and Elaidic acid reached 256% compared to control when 0.1 M by glycerol added to culture medium.

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Compliance with Ethical Standards: This article does not contain any studies with human or animal subjects.

Conflict of Interest: The authors declare that they have no conflict of interest.

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