Effect of nutrients and gamma radiation on growth and lipid accumulation of *Chlorella vulgaris* for biodiesel production

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**ABSTRACT**

The present work investigated the effects of nitrogen (N), phosphorus (P), carbonate (CO$_3$) concentrations and gamma radiation doses on growth and lipid production of *Chlorella vulgaris*. The obtained results showed that culture conditions have highly significant effects on biomass and lipid accumulation. The maximum lipid content was observed at 350, 40, 150, 20 mg L$^{-1}$ of N, P, Mg, and CO$_3$ concentrations, respectively. While the highest biomass yield was achieved at high N and P as well as reduced Mg and CO$_3$ concentrations. Whereas gamma radiation showed a negative effect. Lipid profile recorded maximum saturated (SFAs, 62.44%) and unsaturated fatty acids (UFAs, 37.56%) with palmitic acid (C16:0), linoleic acid (C18:2), pentadecanoic acid (C15:0), palmitoleic acid (C16:1) and oleic acid (C18:1) as predominant fatty acids, which prove that *Chlorella vulgaris* is a promising feedstock for biodiesel production.

**1. Introduction**

The high rate of population increase worldwide and the development in all life sectors let to large, unacceptable consumption of energy. Fossil fuels are considered non-renewable and unsustainable energy because of depleting resources, fluctuation of price and cause global warming (Schenk et al., 2008). Recently, the world has focused on the utilization of renewable and sustainable sources of energy to replace the fossil (Ameen et al., 2019).

Microalgae are the promising feedstock for biofuels needed in all life sectors due to their high growth rates and lipid contents (Rittmann, 2008). Moreover, lipid productivity of several microalgal species was reported to be significantly exceeded those produced by all oil seeded crops (Chisti, 2007). Macronutrients (N, P, Mg, and CO$_3$) are mostly required for all algal vital processes (Klok, Lamers, Martens, Draaisma, & Wijffels, 2014; Zhang, Zhang, Zhuang, & Zhou, 2014). Components of culture media largely affect algal growth and lipid content (Li, Horsmand, Wang, Wu, & Lan, 2008).

Under nutrients and environmental stresses; algae alter their metabolism to produce hydrocarbons (lips and/or carbohydrates). This defense mechanism has been used extensively for biodiesel production (Yu et al., 2016).

Nitrogen starvation (Rehman & Anal, 2019), phosphate limitation (Yu et al., 2016), magnesium supplementation (Esakkimuthu, Krishnamurthy, Govindarajan, & Swaminathan, 2016; Goh et al., 2019), carbon source (Raeiosssadadi, Ahmadzadeh, McHenry, & Moheimani, 2014), iron content of the culture medium (Chandra, Amit & Ghosh, 2019), high salinity (Ji et al., 2018), and high light intensities (He, Yang, & Hu, 2018) can enhance the lipid content. Gamma radiation had a significant effect on the biochemical composition of phytoplankton, although these effects were species-specific (Golz & Bradshaw, 2019). The growth density of *Chlorella pyrenoidosa* mutated by 500 Gy of 60Co irradiation increased by 53.1% (Cheng et al., 2013). While at 900 Gy of 60Co irradiation, the lipid content of *Nitzschia* sp. decreased to 9.9%, whereas the lipid yield increased to 19.76 mg L$^{-1}$ (Cheng et al., 2014).

However, there is limited information available on the effect of gamma radiation on microalgal species, making environmental radioprotection of this group of species challenging, as well as leaving a significant gap of information for these species (Gomes et al., 2017). Generally, microalgae when exposing to stress conditions it shifts their metabolism, instead of synthesis new cell materials, it accumulates lipids and carbohydrates (Hu, 2004). In this study, the effect of nutrients concentrations (N, P, Mg, and CO$_3$) and gamma radiation doses on the algal biomass as well as lipid production of the green microalga, *Chlorella vulgaris* as a promising feedstock for biodiesel were studied.

**2. Materials and methods**

**2.1. Algal species and culturing**

The green alga, *Chlorella vulgaris* used in this work was provided from the Department of Botany and
2.2. Effect of nutrient concentration on growth and lipid accumulation of C. vulgaris

For all experiments, the alga was grown in BG-11 medium and incubated under the previously mentioned conditions. The effect of nitrogen (N) (0, 380, 750, 1500, and 3000 mg L⁻¹, using NaNO₃ as nitrogen source), phosphorus (P) (0, 40, 80, 160, and 320 mg L⁻¹, utilizing K₂HPO₄ as phosphorus source), magnesium (Mg) (19, 38, 75, 113 and 150 mg L⁻¹, serving MgSO₄·7H₂O as magnesium source) and sodium carbonate (CO₃) (0, 10, 20, 40 and 80 mg L⁻¹; using Na₂CO₃ as CO₃ source) concentrations on algal biomass and lipid content of C. vulgaris was determined separately. The growth was monitored every 5 days by optical density (OD680). The lipid content was determined at the end of the incubation period (25 days).

2.3. Effect of gamma radiation on growth and lipid accumulation of C. vulgaris

C. vulgaris Cells in 50 mL aliquots [OD680 = 0.54] were irradiated with 0, 25, 50, 100, 200, 300, 500, 1000 Gy of ⁶⁰Co γ rays. The irradiation was carried out at National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Egypt) using ⁶⁰Co γ rays (Gamma cell 4000-A, India) at a dose rate of 1.296 KGY/h. The irradiated cells were inoculated in 1 L flasks of culture medium and incubated under the previously used conditions. The growth was monitored every 5 days by optical density (OD680). The lipid content was calculated at the end of the exponential growth phase (25 days).

2.4. Growth and lipid measurements

2.4.1. Cell growth measurement

Three mL of culture was sampled every 5 days in triplicates and the cell growth (OD680) was estimated spectrophotometrically using UV-Vis spectrophotometer (T60, UK). The cell dry weight (D.W.) was measured gravimetrically where 20 mL of sample was filtered through pre-weighed filter paper (0.45 μm) and washed with deionized water. The filtered cells were placed on the oven at 60°C for 24 hrs, cooled then weighed. The D.W. was expressed as g L⁻¹.

The specific growth rate, μ_max (d⁻¹), was calculated as (Levasseur, Thompson, & Harrison, 1993):

$$\mu_{\text{max}} = \frac{1}{t} \ln \frac{X_t}{X_0}$$  \hspace{1cm} (1)

Where X_t and X_0 are the biomass concentration (g L⁻¹) at the end and beginning of the experiment respectively, and t is the incubation period (days).

The biomass productivity (BP) (mg L⁻¹·d⁻¹) and biomass yield (BY) were calculated as follows (Vidyashankar et al., 2015):

$$BP = \frac{(X_t - X_0)}{(T_2 - T_1)}$$  \hspace{1cm} (2)

$$BY = \frac{(X_t - X_0)}{X_0}$$  \hspace{1cm} (3)

Where X_t and X_0 are biomass concentrations (g L⁻¹) at the end and beginning of a batch run, respectively; T₁ is the initial time i.e. day 0 and T₂ the final day of incubation.

2.4.2. Lipids measurement

Extraction of lipids was carried out using Bligh and Dyer (1959) method, where 0.3 g of dried algal biomass was extracted by a mixture of chloroform/methanol/deionized water (1:1:0.9). The mixture was filtered to remove the algal pellets. The chloroform layer containing lipid was separated and the solvent was evaporated (40–45°C). The extracted lipids were weighed to give lipid content as the percentage of the cell D.W:

$$L = \frac{W_l}{W_b} \times 100$$  \hspace{1cm} (4)

Where L is the lipid content (%), W_l and W_b are the lipids and the dry biomass weights, respectively.

Lipid productivity (LP) (mg L⁻¹·d⁻¹) was calculated as follows (Hempel, Petrick, & Behrendt, 2012):

$$LP = BP \times L$$  \hspace{1cm} (5)

Lipid yield (LY) (g L⁻¹) was calculated as follows (Yang et al., 2014):

$$LY = BY \times L$$  \hspace{1cm} (6)

2.4.3. Fatty acids analysis

Extracted lipids were transesterified according to Christie (1993). The fatty acid analysis was performed by gas chromatography (Perkin Elmer Auto System XL) equipped with a flame ionization detector and...
a DB5silica capillary column (60 m × 0.32mm i.d.). The oven temperature was maintained initially at 45°C and then programmed to 60°C at a rate of 1°C/min, before finally programmed from 60°C to 240°C at a rate of 3°C/min. Helium was used as the carrier gas at the flow rate of 1 ml min$^{-1}$. The injector and the detector temperatures were set at 230°C and 250°C, respectively.

2.5. Statistical analysis

One-way ANOVA with 95% confidence ($p < 0.05$) was used to determine the significant difference independent variables. The differences between levels were identified by Tukey’s test. The statistical analysis were performed using Minitab software (V18, Minitab Inc., State College, PA, USA). All the experiments were carried out in triplicate.

3. Results and discussion

3.1. Effect of nutrients and gamma radiation on growth of C. vulgaris

The effects of initial concentrations of N, P, Mg, CO$_3$ and gamma radiation doses on the growth (OD) of C. vulgaris were represented in Figure 1. Maximum growth was obtained at 1500, 160, 75, 20 mg L$^{-1}$ of N, P, Mg, CO$_3$, respectively. Elevated concentrations of N and P induced an enhancement of biomass production (Figure 1(a,b)). In contrast, the reduced concentrations of Mg and CO$_3$ stimulated the growth of the studied alga more than the highest concentrations (Figure 1(c,d)). While gamma radiation doses showed neither stimulatory nor inhibitory effects on algal biomass (Figure 1(e)).

![Figure 1](image_url)

Figure 1. Growth curves of C. vulgaris under different concentrations of nitrogen (a), phosphorus (b), and magnesium (c), CO$_3$ (d) and gamma radiation doses (e). Error bars represent ±SD of three replicates.
3.2. Effect of nutrients and gamma radiation on biomass and lipid accumulation of C. vulgaris

3.2.1. Nitrogen

The algal biomass and lipid accumulation of C. vulgaris were significantly affected by nitrogen concentration ($p < 0.05$) as illustrated in Figure 2(a,b). The highest biomass productivity and yield of C. vulgaris (40.41 ± 4.29 mg L$^{-1}$d$^{-1}$ and 1.10 ± 0.11 g L$^{-1}$), respectively were achieved at 3000 mg L$^{-1}$ of N. Gradually increase in nitrogen concentration (0–3000 mg L$^{-1}$) led to an obvious enhancement in specific growth rate and biomass yield, which was accompanied by retardation in lipid content. The maximum lipid content of 21.47 ± 0.65% was recorded under nitrogen limitation (380 mg L$^{-1}$).

Lipid productivity and yield were affected directly by the growth rate. The maximum lipid productivity and lipid yield of 7.05 ± 0.47 mg L$^{-1}$d$^{-1}$ and 0.187 ± 0.011 g L$^{-1}$ were obtained at a 1500 mg L$^{-1}$ of N, respectively.

Economically, nitrogen-starvation was the strategy mostly adopted for lipid production from microalgae as the most practical way on the large scale (Ramya, Ambily, Sujitha, Arumugam, & Maiti, 2017). Furthermore, nitrogen deprivation during 4 to 9 days in culture media, induced marked enhancement in lipid content (2 – 3-folds) in green microalgae (Sajjadi, Chen, Raman, & Ibrahim, 2018). Our results showed an agreement with the results obtained by Li et al. (2013) who illustrated that the nitrogen deficiency reduces the growth rate but enhances the lipid biosynthesis in microalgae. Also, our results were in Coincidence with those of Feng, Deng, Fan, and Hu (2012) who reported an augmentation in lipid content of Chlorella zofingiensis and the maximum lipid productivity (87.1 mg L$^{-1}$ d$^{-1}$) resulted from nitrogen-deficiency in growth media. Similarly, Rehman and Anal (2019) observed an increase in the lipid of Chlorococcum sp. TISTR from 17.05% to 29.59% when it was cultivated on nitrogen limitation medium and optimized light intensity.

3.2.2. Phosphorus

The influence of phosphorus concentrations is illustrated in Figure 3(a,b). From the statistical point of view (ANOVA), the results showed that the produced biomass and lipid of the studied alga were significantly affected by the variation in phosphorus concentration ($p < 0.05$). The maximum specific growth rate (0.116 ± 0.004 d$^{-1}$) and biomass productivity (43.25 ± 5.70 mg L$^{-1}$d$^{-1}$) and biomass yield (1.14 ± 0.146 g L$^{-1}$) were obtained at a 1500 mg L$^{-1}$ of P. Elevation of the phosphorus levels from 40 to 3000 mg L$^{-1}$ led to an obvious enhancement in specific growth rate and biomass yield, which was accompanied by retardation in lipid content.

Figure 2. Biomass yield, lipid yield, lipid content, specific growth rate, biomass productivity and lipid productivity of C. vulgaris cultured in different nitrogen concentrations. Different small letters on the lines and bars indicate significant difference ($p<0.05$). Error bars represent ± SD of three replicates.
320 mg L\(^{-1}\) has no significant impact on the biomass \((p > 0.05)\), but the lipid content and productivity decreased. The highest lipid content (18.26 ± 0.21\%) and lipid productivity (7.05 ± 0.47 mg L\(^{-1}\) d\(^{-1}\)) were achieved at 40 mg L\(^{-1}\) of P, respectively.

Phosphorus is considered a vital nutrient for microalgal growth as well as it has a very important role in the metabolic processes. The obtained results are in agreement with those of Spijkerman and Wacker (2011) who recorded an increase in total fatty acids content up to under phosphorus depletion, while increasing phosphorus concentration in media led to a decrease over a factor of ten in the total fatty acids content. Additionally, under phosphorus deprivation, the recorded cell division and photosynthesis rates were decreased. This may lead to an enhancement of triacylglycerols accumulation which was rich in monounsaturated and saturated fatty acids (Guschina & Harwood, 2009). Similarly to other reported studies, the highest lipid content of *Chlorella* sp. (Liang, Zhang, Gu, & Cong, 2013) and *Phaeodactylum tricornutum* (Yu et al., 2016) was achieved under phosphorus limitation environment. In another study, the biomass of *Chlamydomonas reinhardtii* CC124 was reduced by up to 31.7%, although lipid content in cells was significantly increased (Yang et al., 2018).

### 3.2.3. Magnesium

Figure 4(a,b) shows the effects of initial magnesium concentration. The obtained results demonstrated that low and high Mg concentrations have a negative impact on algal biomass. The maximum specific growth rate (0.113 ± 0.004 d\(^{-1}\)) and biomass productivity (38.65 ± 2.58 mg L\(^{-1}\) d\(^{-1}\)) were recorded at 75 mg L\(^{-1}\) of Mg. Cellular lipid content was maximally increased up to 20.77 ± 0.7\% at 150 mg L\(^{-1}\) of Mg. While the maximum lipid productivity and yield of 7.05 ± 0.47 mg L\(^{-1}\) d\(^{-1}\) and 0.187 ± 0.012 g L\(^{-1}\) were observed at 75 mg L\(^{-1}\) of Mg, respectively.

Magnesium is essential for algal growth, not only because of constituting central atom of chlorophyll but also has an important role in the metabolic pathways as co-factor of certain enzymes (Wang et al., 2014). The key role of Mg is catalyzing the initial step of the biosynthesis of fatty acid (Nelson & Cox, 2008). Mg starvation is expected to decrease the rate of cell division, hinder the synthesis of chlorophyll and consequently, reduce the biomass yield (Finkle & Appleman, 1953). However, there are limited investigations on responses of algae
through magnesium deficiency regarding growth and lipid production (Goh et al., 2019). Esakkimuthu et al. (2016) reported an increase in total lipid (54.6%) of Scenedesmus obliquus under magnesium supplementation which is in agreement with our results. Additionally, $2.4 \times 10^{-3}$ g L$^{-1}$ of Mg led to a significant increase in the lipid content and biomass of algae (Sydney et al., 2010).

Increasing Mg concentration induced an amelioration in biomass of both C. vulgaris and S. obliquus. At 150 mg L$^{-1}$ of Mg, the S. obliquus and C. vulgaris biomass was increased to 36 and 33%, respectively on day 18th of cultivation. While, at 100 mg L$^{-1}$ of Mg the lipid content was reached 27% and 26%, respectively On 15th day of cultivation (Gorain, Bagchi, & Mallick, 2013).

3.2.4. Carbonate

High and low levels of carbonate were shown to induce a significant impact ($p < 0.05$) on the biomass and lipid accumulation (Figure 5(a,b)). At 20 mg L$^{-1}$ of CO$_2$, the highest biomass yield (1.03 ± 0.06 g L$^{-1}$) and lipid content (18.26 ± 0.21%) were achieved. Whereas, increasing carbonate concentration led to remarkable and significant retardation in biomass productivity and growth rate. High lipid productivity (7.05 ± 0.47 mg L$^{-1}$d$^{-1}$) and yield (0. 187 ± 0.012 g L$^{-1}$) were recorded on culturing with 20 mg L$^{-1}$ of CO$_2$. Elevation of carbonate concentration from 20 to 80 mg L$^{-1}$ showed a negative effect on the biomass and lipid accumulation.

Most recent works investigated the impact of inorganic carbon supplementation and lipid accumulation in microalgal species have focused on CO$_2$ as a carbon source (Chiu et al., 2009). In other studies, sodium bicarbonate was utilized as an inorganic carbon source for studying algal growth and metabolites in various microalgal species. Furthermore, it has been found that triacylglycerol accumulation (TAG) was improved under sodium bicarbonate addition (Piromrat, Direkbusarakom, Chinajariyawong, & Powtongsook, 2010). In agreement with our results, Gardner et al. (2012) recorded an increase in the TAG level of Phaeodactylum tricornutum Pt-1 and Scenedesmus sp. WC-1 under bicarbonate supplementation. Whereas, Zhao, Yu, Jiang, Zhang, & Tan (2012) found that the lipid content of Scenedesmus quadricauda increased using air while the incorporation of sodium bicarbonate in the growth medium had a negative impact on the lipid biosynthesis. Moreover, supplementation of medium with 0.6 g/L of NaHCO$_3$ led to an increase of up to 20.91% in the lipid content as well as increased biomass concentration of Scenedesmus sp. CCNM 1077 compared to normal growth conditions as reported by Pancha et al. (2015).

Figure 4. Biomass yield, lipid yield, lipid content, specific growth rate, biomass productivity and lipid productivity of C. vulgaris cultured in different magnesium concentrations. Different small letters on the lines and bars indicate significant difference ($p<0.05$). Error bars represent ± SD of three replicates.
In addition, the influence of carbonate and bicarbonate on the algal growth and lipid production of *Scenedesmus quadricauda* was investigated. The highest biomass yield of 0.29 g L$^{-1}$ and 0.225 g L$^{-1}$ was obtained at 0.5 g L$^{-1}$ Na$_2$CO$_3$ and 2 g L$^{-1}$ NaHCO$_3$ after 15 days of the incubation period. While, the highest lipid yield of 0.198 g L$^{-1}$ and 0.163 g L$^{-1}$ was observed at 1.5 g L$^{-1}$ Na$_2$CO$_3$ and 2 g L$^{-1}$ NaHCO$_3$, respectively after 3 days of the incubation period (Anusree, Sulochana, Javee, & Muthu, 2017).

### 3.2.5. Gamma radiation

Algal growth parameters were shown to be inversely proportional to gamma radiation doses (Figure 6(a,b)). The biomass yield was decreased drastically from 1.03 ± 0.06 g L$^{-1}$ to 0.10 ± 0.01 g L$^{-1}$ (by 90.29%) when cultures were exposed to irradiation dose of 1000 Gy. These results indicated that the high doses of gamma radiation (γ-ray) had a significantly negative effect on microalgal cell growth. Hence, increasing the radiation dose from zero to 1000 Gy induced a significant change in the lipid content ($p < 0.05$). Maximum lipid content (18.26 ± 0.21%), lipid productivity (7.05 ± 0.47 mg L$^{-1}$d$^{-1}$) and yield (0.187 ± 0.012 g L$^{-1}$) were recorded at zero Gy. Compared with the 90.29% decrease in biomass, the decrease in lipid content was relatively small (34.67%).

Gamma radiation has a biological impact on the constituents of the cell, especially water molecules. Gamma rays interact with growth media and generate free radicals, which can alter cell composition, which can cell composition (Kovacs & Keresztes, 2002), these findings agree with our results. Nevertheless, when the microalgal cells were exposed to high doses of γ-rays, the cells disintegrated, or broken down, if it loses their ability to self-repair and failed to recover completely (Agarwal, Rane, & Sainis, 2008; Kovacs & Keresztes, 2002). Whereas under low irradiation doses, cells were still slightly damaged and within a short time the cells recovered to normal levels (Fuma et al., 2009). Furthermore, protein synthesis was noted to decrease with increasing irradiation dose, which may lead to an enhancement of the photosynthetic photoinhibition (Agarwal et al., 2008). On the contrary, the system of cell metabolism regulation was damaged under the high irradiation doses (Agarwal et al., 2008).

Concerning lipid accumulation, firstly the lipid content of microalgal cells was decreased because the lipid biosynthesis was repressed during the recovery of cell metabolism. Secondly, under high irradiation doses, a great number of free radicals were generated which
break down the double bonds of polyunsaturated fatty acids (PUNSFAs) of the biomembrane causing its lipid peroxidation (Agarwal et al., 2008; Cheng et al., 2013). The lipid content of *Nitzschia* sp. was decreased with elevated irradiation dose (0–900 Gy) (Cheng et al., 2014), which went parallel with obtained results in this investigation.

### 3.3. Fatty acid composition

The lipid profile of *C. vulgaris* was given in Table 1. The fatty acid composition showed the presence of twelve identified fatty acids. Moreover, fatty acids methyl esters (FAMES) mostly contains saturated (SFAs, 62.44%) and unsaturated (UFAs, 37.56%) fatty acids with carbon chain lengths from C12 to C24. The predominant component fraction is palmitic acid (C16:0, 42.40%) followed in descending order by linoleic acid (C18:2, 20.30%), pentadecanoic acid (C15:0, 11.40%), palmitoleic acid (C16:1, 8.30%) and oleic acid (C18:1, 5.20%). Meanwhile, the percentage of other fatty acids was relatively low. Also, the amount of monounsaturated fatty acid (MUSFAs) and polyunsaturated fatty acid (PUSFAs) were 13.5 and 24.06%, respectively.

Concerning the biodiesel production from *C. vulgaris*, the fatty acids profile of green microalgae is similar to vegetable oils, whereas it contains mainly C16 and C18 fatty acids, which are compatible with biodiesel (Converti, Casazza, Ortiz, Perego, & Del Borghi, 2009; Francisco, Neves, Jacob-Lopes, & Franco, 2010). Generally, the increasing chain length will lead to an increase in the heat of combustion, viscosity and cetane number, which means that C16–18 fatty acids are desirable for biodiesel (Francisco et al., 2010). Moreover, the obtained results showed that 80.78% of

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**Table 1. Lipid profile of *C. vulgaris* cultured on BG11 medium.**

<table>
<thead>
<tr>
<th>Type of fatty acid</th>
<th>Fatty acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric acid (C12:0)</td>
<td>1.12</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>2.90</td>
</tr>
<tr>
<td>Pentadecanoic acid (C15:0)</td>
<td>11.40</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>42.40</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)</td>
<td>8.30</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>0.82</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>5.20</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>20.30</td>
</tr>
<tr>
<td>Linolenic acid (C18:3)</td>
<td>3.76</td>
</tr>
<tr>
<td>Arachidic acid (C20:0)</td>
<td>0.40</td>
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<tr>
<td>Behenic acid (C22:0)</td>
<td>1.10</td>
</tr>
<tr>
<td>Lignoceric acid (C24:0)</td>
<td>2.30</td>
</tr>
<tr>
<td>Saturated fatty acid (SFAs)</td>
<td>62.44</td>
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<td>Unsaturated fatty acid (USFAs)</td>
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</tr>
<tr>
<td>Monounsaturated fatty acid (MUSFAs)</td>
<td>13.5</td>
</tr>
<tr>
<td>Polyunsaturated fatty acid (PUFAs)</td>
<td>24.06</td>
</tr>
</tbody>
</table>

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Figure 6. Biomass yield, lipid yield, lipid content, specific growth rate, biomass productivity and lipid productivity of *C. vulgaris* cultured in different gamma radiation doses. Different small letters on the lines and bars indicate significant difference (*p* < 0.05). Error bars represent ± SD of three replicates.
fatty acids *C. vulgaris* was C16–18 which may give the best relation between cold flow properties and stability against oxidation (Knothe, 2009). MUFA s are commonly composed of palmitoleic acid (16:1) and oleic acid (18:1) which are the most suitable and frequent fractions for biodiesel production (Knothe, 2011).

The C16- C18 content of *C. vulgaris* is markedly higher than in *Scenedesmus obliquus* CNW-N (67–86%) (Ho, Chen, & Chang, 2010), and closer to *Haematococcus pluvialis* (76.6%) (Damiani, Popovich, Constenla, & Leonardi, 2010). The produced lipid is mainly consisting of unsaturated fatty acids (UFAs) and saturated fatty acids (SFAs). The high USFAs content in microalgal lipid will improve cloud point and lubricity properties for produced biodiesel (Knothe, 2005). In addition, Song et al. (2014) have observed an elevated percentage of fatty acids (SFAs ~ 50.16% and MUFA s ~ 48.79%). These findings propose that *C. vulgaris* lipid could perform low iodine value and high cetane number which meet with the US (ASTM D6751) and European (EN 14214) standards requirements (Hoekman, Broch, Robbins, Ceniceros, & Natarajan, 2012).

Additionally, The FAMEs profile contains a remarkable percentage of polyunsaturated fatty acids (PUFAs). Based on the European standard (EN 14214) for biodiesel, the polyunsaturated fatty acids (≥3 double bonds) should be 1%, which could affect the properties of biodiesel (Branco-Vieira et al., 2017). *C. vulgaris* demonstrated a considerable amount of C18:2 and C18:3, resulting in low melting points, also are appropriate for the low temperature of biodiesel (Knothe, 2005).

### 4. Conclusion

The effect of macronutrients and gamma radiation on the growth parameters and lipid production of *C. vulgaris* were studied. The maximum biomass was recorded under nitrogen and phosphorus depletion as well as reduced magnesium and carbonate. While the gamma radiation showed a negative impact on both biomass and lipid content. The lipid content was enhanced pronouncedly with increasing the concentration of Mg and reducing N, P and carbonate concentration. Palmitic acid (C16:0) and linoleic acid (C18:2) were dominated Constituents the algal fatty acids (62.7% of the total fatty acids), which strongly proved that *C. vulgaris* is a good candidate for biodiesel production.

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### Disclosure statement

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