Impact of Culture Media Composition, Nutrients Stress and Gamma Radiation on Biomass and Lipid of the Green Microalga, *Dictyochloropsis splendida* as a Potential Feedstock for Biodiesel Production

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**Abstract:**

Biodiesel production from microalgae depends on the biomass and lipid production. Both biomass and lipid accumulation is controlled by several factors. The effect of various culture media (BG11, BBM, and Urea), nutrients stress [nitrogen (N), phosphorous (P), magnesium (Mg) and carbonate (CO₂)] and gamma (γ) radiation on the growth and lipid accumulation of *Dictyochloropsis splendida* were investigated. The highest biomass and lipid yield of *D. splendida* were achieved on BG11 medium. Cultivation of *D. splendida* in a medium containing 3000 mg L⁻¹ N, or 160 mg L⁻¹ P, or 113 mg L⁻¹ Mg, or 20 mg L⁻¹ CO₃, led to enhanced growth rate. While under the low concentrations of nutrients caused a marked increase in the lipid content. Cultures exposure to 25 Gy of γ-rays, led to an increase in lipid content up to 18.26 ± 0.81 %. Lipid profile showed the maximum presence of saturated fatty acids (SFAs, 63.33%), and unsaturated fatty acids (UFAs, 37.02%). Fatty acids (FAs) recorded the predominance of C16:0, C18:2, C15:0 and C16:1, which strongly proved *D. splendida* is a promising feedstock for biodiesel production.

**Keywords:** Biodiesel, *Dictyochloropsis splendida*, Gamma radiation, Lipid content, Nutrients.

**Introduction:**

Renewable, sustainable, and eco-friendly biofuels are development fields and attractive research that are much needed because of fossil fuels depletion and environmental pollution. Biodiesel has several advantages such as high biodegradable, absence of any aromatic compounds and 90% reduction in air toxicity may conduct to 95% decrease in the applicable cancer cases and have similar properties of fossil diesel.

Biodiesel can be classified according to their source into 1) biodiesel produced from edible oil (first generation) such as soybeans, rapeseed, and sunflower seeds. About 7% of global edible vegetable oil supplies were utilized for biodiesel production in 2007. However, vast use of edible oils may cause food supplies versus fuel issue (food crisis). 2) biodiesel produced from waste cooking oil, animal fats and nonedible vegetable oils (second generation) such as jatropha, and 3) third generation biodiesel is produced from microalgae.

The advantages of microalgae over higher plants as a source of biodiesel: 1) synthesize and accumulate large quantities of neutral lipids, 2) Possess a high photosynthetic efficiency and growth rate, 3) Grow on saline/brackish water and non-arable land as well as it can utilize nitrogen (N) and phosphorous (P) of wastewater, 4) Can grow in photobioreactors with higher biomass production. 5) Sequester CO₂ through photosynthesis and so reducing greenhouse gas emission.

Current research into increasing lipid accumulation in microalgal cells mainly focuses on the optimization of culture conditions, screening microalgae species, and the transformation of microalgae by genetic engineering. Limitation of nutrients in culture media is a commonly technique
used to increase lipid inside the microalgal cells. N and P starvation besides magnesium (Mg) and carbon supplementation can induce biosynthesis of FAs.8,9

Little information is available on the effect of γ-radiation on the physiological mechanism and biochemical composition of microalgae.10,11 The objectives of this study were to investigate the effect of culture media composition, nutrients concentration (nitrogen, phosphorous, magnesium, and carbonate) as well as the dosage of γ-radiation on both algal growth and lipid parameters of microalga, Dictyochloropsis splendida

Materials and Methods:
Cultivation of microalgae
The green alga, Dictyochloropsis splendida was provided by the algal culture collection from the Laboratory of Phycology in Botany and Microbiology Department, Faculty of Science, Cairo University, Egypt. The alga was cultivated on BG-11 medium12 and incubated under a continuous light intensity of 40 µE m⁻² s⁻¹ (daylight fluorescent lamps, Philips, TLD18W/54-765) at 25± 1°C and aeration with constant sterilized bubbling of air (by a 0.22µm filter) for 25 days.

Influence of media composition on growth and lipid production of D. splendida
To evaluate the impact of media composition on the growth and lipid content, D. splendida was cultured in 1L glass flasks in BG11 medium12, BBM13 and urea medium14. Flasks were incubated under the same previous conditions.

Influence of nutrients concentrations on growth and lipid production of D. splendida
In all experiments, D. splendida was grown in BG11 medium12 under continuous illumination with aeration rate of 1.25 L/min at 25± 1°C for 25 days. Nitrogen was used in the form of NaNO₃ in concentrations 0, 380, 750, 1500, and 3000 mg L⁻¹. Phosphorous (P) was used in the form of K₂HPO₄ in concentrations of 0, 40, 80, 160, and 320 mg L⁻¹. Magnesium (Mg) was used as MgSO₄•7H₂O in concentrations 19, 38, 75, 113 and 150 mg L⁻¹. Carbonate (CO₃) was used as Na₂CO₃ in concentrations of 0, 10, 20 40 and 80 mg L⁻¹. Growth parameters and lipid content were determined at each experiment.

Influence of γ-radiation on growth and lipid production of D. splendida
Cultures of D. splendida were irradiated by different γ-doses 0, 25, 50, 100, 200,300, 500, 1000 Gy of ⁶⁰Co γ-rays. Irradiation was performed by ⁶⁰Co γ-rays (Gamma cell 4000-A- India) at National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Egypt at a dose rate of 1.296 KGy/h. Cultures after irradiation were incubated under previous conditions and growth was determined as optical density at 680 nm. The lipid content was calculated at the end of the experiment.

Cell growth measurements
From 1L incubated algal culture (900 ml BG11 + 100 ml algal inoculum), optical density (OD at 680nm) of the microalgal sample (3ml) was determined at regular interval of 5 days (in triplicates) using spectrophotometer (UV–Vis spectrophotometer, T60, UK). Twenty ml of washed filtered culture were dried at 105°C for 24 hrs., chilled in a desiccator, and the algal dry weight was determined and expressed as g L⁻¹. The maximum specific growth rate, μmax (d⁻¹), was evaluated as:

$$\mu_{\text{max}} = \frac{1}{t} \ln \frac{X_f}{X_0} \quad \text{... ... ... (1)}$$

Where $X_f$ and $X_0$ are the biomass concentrations (g L⁻¹) at the final and the start of a batch run, respectively; and $t$ is the time span of the run (day). The biomass productivity ($BP$) (mg L⁻¹d⁻¹) and biomass yield ($BY$, g L⁻¹) were assessed as follows15:

$$BP = \frac{(X_f - X_0)}{(T_2 - T_1)} \quad \text{... ... ... (2)}$$

$$BY = \frac{(X_f - X_0)}{X_f} \quad \text{... ... ... (3)}$$

Where $X_f$ and $X_0$ are the biomass concentrations (g L⁻¹) at the final and the start of a batch run, respectively; and $T_1$ and $T_2$ (day) represent the incubation time of an experiment at the start time day and the final day of incubation, respectively.

Determination of lipid content
Lipids were extracted at the final incubation time by a 1:1:0.9 ratios of chloroform: methanol: deionized water mixture on volumetric basis16 where 5 ml chloroform, 10 ml methanol, and 4 ml of deionized water were initially added to 0.3 g dried sample (0.3 g dried algal biomass/1L algal culture). Then, the mixture was shaken for 10 min, and then another 5 ml chloroform and 5 ml deionized water were added and shaken for overnight. The algal-solvent mix was refined to eliminate the algal precipitates. The chloroform layer of the filtrate was removed, solvent was volatilized at 40-45°C and the lipid was weighed. Lipid content was determined as percentage of cell dry weight:

$$LC = \frac{W_L}{W_B} \times 100 \quad \text{... ... ... (4)}$$
Where $LC$ is the lipid content ($\%$), $W_L$ and $W_B$ are the weights of the extracted lipids and the dry biomass, respectively.

The lipid productivity ($LP$) was calculated as follows \(^{(5)}\):

\[
LP = BP \times LC \quad \ldots \ldots \quad (5)
\]

Where $LP$ is the lipid productivity (mg L\(^{-1}\)d\(^{-1}\)), $BP$ (mg L\(^{-1}\)d\(^{-1}\)) and $LC$ ($\%$ dry weight) are biomass productivity (BP) and lipid content, respectively.

Lipid yield was calculated as follows \(^{(6)}\):

\[
LY = BY \times LC \quad \ldots \ldots \quad (6)
\]

Where $LY$ is lipid yield (g L\(^{-1}\)), $BY$ (g L\(^{-1}\)), $LC$ ($\%$ dry weight) are biomass yield and lipid content, respectively.

**Transesterification and Fatty acid analysis**

Lipid was transesterified to produce fatty acid methyl ester (FAMEs) using 2% sulphuric acid in methanol \(^{(9)}\). FA analysis was achieved in Central Laboratory, Faculty of Agriculture, El-Azhar University by gas chromatography (Perkin Elmer Auto System XL) using DB5 silica gel capillary column (60 m $\times$ 0.32mm i.d.) with flame ionization detector and Helium was applied as the carrier gas (at the flow rate of 1 ml min\(^{-1}\)).

**Statistical analysis**

All the experiments were conducted in 3 replicates. One-way ANOVA with 95% confidence (probability limit of $p < 0.05$ was utilized to estimate the significant difference in dependent variables, and Tukey’s test at a reliability level of ($p$<0.05) was used to identify differences between each level of treatment. The statistical analyses were achieved using Minitab software (V18, Minitab Inc., State College, PA, USA).

**Results and Discussion**

**Influence of media composition on growth and lipid accumulation**

The effect of various culture media (BG11, BBM and Urea) composition on the growth of *D. splendida* were assessed as outlined in Figure 1. The highest $BY$ of *D. splendida* ($0.90 \pm 0.01$ g L\(^{-1}\)) resulted in culturing on BG11 medium. With this medium, the maximum $\mu_{max}$ and $BP$ were $0.097 \pm 0.002$ d\(^{-1}\) and $32.96 \pm 0.54$ mg L\(^{-1}\)d\(^{-1}\), respectively. Also, highest $LC$, $LB$ and $LY$ were $16.92 \pm 0.07$ %, $5.58 \pm 0.07$ mg L\(^{-1}\)d\(^{-1}\) and $0.152 \pm 0.001$ g L\(^{-1}\), respectively while urea medium showed the lowest $LC$ ($10.43 \pm 0.79$ %) as illustrated in (Figure 2A,B). The increase in the $LY$ of *D. splendida* when cultured on the BG11 medium may be back to the high N concentration ($1.5$ g L\(^{-1}\)) in the BG11 medium which led to an increasing $\mu_{max}$ where $LY$ is the product of the $BY$ multiplied by the $LC$ \(^{(18)}\). This finding went parallel with Chandra et al \(^{(20)}\) who studied the effect of different culture media (BG-11, modified CHU-13 and BBM medium) on the growth and lipid production of *Chlorella minutissima*. Maximum $BY$ and $LY$ were achieved by modified CHU-13 medium ($970 \pm 0.21$ and $356.63 \pm 0.51$ mg L\(^{-1}\), respectively) succeed in descending order by those produced by BG-11 medium ($850 \pm 0.12$ mg L\(^{-1}\) and $243.65 \pm 0.30$ mg L\(^{-1}\), respectively) and the minimum values were recorded by BBM medium ($730 \pm 0.42$ mg L\(^{-1}\) and $196.83 \pm 0.43$ mg L\(^{-1}\), respectively).

In another study, *Chlorella* sp. and *Scenedesmus* sp. were cultivated in media with more or less nutrients. Accumulation of lipid was higher in media deficient of nutrients whereas $\mu_{max}$ and $LP$ were reduced \(^{(21)}\). Furthermore, micronutrients such as iron, cobalt, zinc, copper and manganese and nickel are the most essential trace metals required by algae for several metabolic functions \(^{(22)}\). This supports our results, where the highest $\mu_{max}$ and $LP$ of *D. splendida* were recorded on BG11 medium followed in descending order by BBM and urea medium, which may be due to the availability (or not) of nutrients in the media \(^{(23)}\). On other hand, several studies used nitrate in source of N in culture media, whereas urea has been highly applied in large-scale algal cultivation due to its competent low cost compared to the others. Nevertheless, the manipulation of urea concentration through the cultivation is the challenge. Urea can liberate urease or be hydrolyzed to ammonia in basic conditions which lead to the growth of inhibition at high levels \(^{(24)}\).

Figure 1. Growth curve of *D. splendida* cultured on different culture media. Error bars represent ±SD of three replicates.
Cell density increased with an increase in initial P concentration. From the ANOVA results, we found that P had a remarkable effect \((p<0.05)\) on biomass production of \(D.\ splendida\). The maximum \(\mu_{max}\::BP\) and \(BY\) of \(0.111 \pm 0.010 \, \text{d}^{-1}\), \(41.01 \pm 3.96 \, \text{mg L}^{-1}\cdot\text{d}^{-1}\), and \(1.10 \pm 0.11 \, \text{g L}^{-1}\) were obtained at 160 mg \(\text{L}^{-1}\), respectively. Increasing the P concentration from 40 mg \(\text{L}^{-1}\) to 320 mg \(\text{L}^{-1}\) had an insignificant effect \((p>0.05)\) on \(BY\) (Table 1). The lipid accumulation of \(D.\ splendida\) under different initial P concentrations was given in Figure 4B. While deficiency in P significantly promoted lipid accumulation \((p<0.05)\). The highest \(LC\) \((18.39 \pm 1.22 \, \%\), \(LP\) \((7.06 \pm 0.82 \, \text{mg L}^{-1}\cdot\text{d}^{-1})\) and \(LY\) \((0.189 \pm 0.023 \, \text{g L}^{-1})\) were recorded at 80 mg \(\text{L}^{-1}\) P as shown in Table 1and Figure 4B.

Phosphorus is the main player in cellular metabolic processes, which are connected to photosynthesis and energy transfer. The results agreed with those of Guschina and Harwood \(^{28}\) who mentioned that under P deficiency, the photosynthetic rates decreased, the cell division rates reduced, and this may lead to the accumulation of triacylglycerols. Also, under P limitation, the \(LC\) of \(Tisochrysis\ lutea\) \(^{29}\) and \(P.\ tricornutum\) \(^{30}\) were increased. In addition, the total FAs content increased over two folds under P depletion, conversely total FAs content was inversely proportional with P concentration over a factor of ten \(^{31}\).

**Magnesium**

Figure 3C illustrates the time-course study on the effect of Mg on the growth of \(D.\ splendida\). Increasing the Mg from 0–75 mg \(\text{L}^{-1}\) showed significant increase \((p<0.05)\) on the growth of \(D.\ splendida\), while increasing Mg from 75 to 150 mg \(\text{L}^{-1}\) had insignificant effect on the \(BY\). The maximum \(\mu_{max}\) and \(BP\) of \(0.120 \pm 0.001 \, \text{d}^{-1}\) and \(34.47 \pm 0.46 \, \text{mg L}^{-1}\cdot\text{d}^{-1}\) were obtained at 113 mg \(\text{L}^{-1}\)

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**Impact of nutrients concentrations on growth and lipid formation**

**Nitrogen**

The impact of initial concentrations of N on the growth of \(D.\ splendida\) was represented in Figure 3A. Increasing the P and N, was accompanied by an increase in growth. The highest \(BP\) and \(BY\) of \(42.06 \pm 2.25 \, \text{mg L}^{-1}\cdot\text{d}^{-1}\) and \(1.15 \pm 0.05 \, \text{g L}^{-1}\), respectively were obtained by cultivation with a start N concentration of 3000 mg \(\text{L}^{-1}\) (Table 1). Elevation of the N concentration from 0 to 3000 mg \(\text{L}^{-1}\) showed an obvious increment in biomass and growth rate, but a decline in lipid accumulation. The highest \(LC\) of \(18.09 \pm 0.03 \, \%\) was recorded under N depletion \((380 \, \text{mg L}^{-1})\) as illustrated in Figure 4A. The \(LY\) of \(D.\ splendida\) was significantly influenced by the N concentration \((P<0.05)\). The highest \(LP\) \((5.37 \pm 0.12 \, \text{mg L}^{-1}\cdot\text{d}^{-1})\) and \(LY\) \((0.0152 \pm 0.001 \, \text{g L}^{-1})\) were recorded at N concentration of 1500 mg \(\text{L}^{-1}\).

Nitrogen is the most commonly reported nutrient-limiting factor in the growth and lipid accumulation of microalgae \(^{24}\). The obtained results agrees with Ishika et al. \(^{25}\) who reported that N deficiency results in an increment in lipid and/or carbohydrate accumulation of microalgae and a decline in growth rate, photosynthetic efficacy, and protein amounts. Rehman and Anal \(^{26}\) noted that the \(LC\) of \(Chlorococcum\ sp.\) TISTR 8583 increased by \(1.7\) folds when cultured on N-deficient medium and optimized light intensity. Similarly, Yodsuwan et al. \(^{3}\) reported that the maximum \(LC\) of \(P.\ tricornutum\) \((53.04 \pm 3.26\% \, \%)\) was noted under N-deficient condition.

**Phosphorous**

The growth curve of \(D.\ splendida\) in the growth medium for different initial P concentrations are shown in Figure 3B. Reasonably, the maximum
Mg, respectively (Table 1). On the contrary, the increasing Mg concentration exhibited a negative impact on the LC. The maximum LC (20.06 ± 0.15 %) was achieved at 19 mg L⁻¹ of Mg (Figure 4C). Further, the LP and LY of the tested microalga were significantly affected by alteration in the Mg concentration (P<0.05).

Mg plays a key role in the growth of microalgae, whereas it is the central atom of chlorophyll and as a co-factor of some enzymes in the metabolic pathway. There are limited studies on microalgae responses during Mg limitation in terms of biomass growth and lipid accumulation. The lipid yield and growth of microalgae were improved by Mg supplementation, whereas the starvation of Mg ions anticipates the decrease in mitotic division, hinder of chlorophyll formation and, so, the biomass yields.

In harmony with the obtained data, Gorain et al. found a marked increase in the neutral lipid content of Chlorella vulgaris and Scenedesmus obliquus in Mg- and Ca-free medium. Also, Increasing the concentration of Mg exhibited positive effects on BY of C. vulgaris and S. obliquus, and at concentration (150 mg L⁻¹) the BY was elevated up to 1.5 g L⁻¹ (36% rise) for S. obliquus and 1.6 g L⁻¹ (33% rise) for C. vulgaris on the 18th day of incubation. While the LC was increased with maximum up to 27% and 26%, respectively at 100 mg L⁻¹ of Mg. The function of Mg ions in switch on the enzyme Acetyl-CoA carboxylase and catalyzing the first stage of FA production was proved. In addition, the productivity of microalgae is augmented when Mg²⁺ concentration is in the range of 2-8 mg/L.

**Carbonate**

High and low sodium carbonate concentration in the growth medium had significant influence (p<0.05) on growth (Figure 3D) and lipid production of D. splendida (Figure 4D). Table 1 summarizes the biomass and lipid parameters of D. splendida under different concentrations of sodium carbonate. At 20 mg L⁻¹ of CO₃, the maximum BY (0.90 ± 0.01 g L⁻¹) was recorded, whereas, rising the CO₃ concentration showed a significant decrease in the growth parameters (µmax and BP) (p<0.05). The highest LC of 19.46 ± 0.32 % was shown at 40 mg L⁻¹ as presented in Figure 4D. The LP and LY ranged between 1.66-5.37 mg L⁻¹d⁻¹ and 0.051-0.152 mg L⁻¹, respectively.

Most investigations that have been done on the effect of inorganic carbon supply and lipid formation in microalgae cultures have converged on the addition of CO₂. In some works, NaH₂CO₃ has been utilized as a source of carbon on experimenting growth and biochemical composition in various microalgae species and induced the accumulation of triacylglycerol in microalgal species. On the contrary, Zhao et al. recorded that the addition of sodium bicarbonate in the culture medium of Scenedesmus quadricauda had a negative influence on the lipid production and the highest LC was obtained under air. On the other hand, Li et al. found that the maximum LC of 494 mg g⁻¹ and LP of 44.5 mg L⁻¹d⁻¹ of C. vulgaris were recorded at 160 mM NaHCO₃ and pH 9.5, and 10 mM NaHCO₃ was the optimal concentration for cell growth and elevating NaHCO₃ from 10 to 160 mM prosecute an inhibition to biomass.

**Gamma radiation**

Figure 3E shows the growth curve of D. splendida under different gamma radiation doses. The data exhibited that high doses of γ-ray had a negative effect on growth. The maximum µmax was decreased with elevating irradiation dose (Table 2). The BY declined from 0.90 ± 0.01 g L⁻¹ to 0.21 ± 0.02 g L⁻¹ (decreased by 76.67 %) when cultures were displayed to irradiation dosage of 1000 Gy. The LC of D. splendida given in Figure 4E, the highest LC of 18.26 ± 0.81 % was achieved when the alga cell exposed to 25 Gy. While the higher irradiation doses had negative impact on the lipid accumulation. The maximum LP (5.37 ± 0.12 mg L⁻¹d⁻¹ and 5.24 ± 0.43 mg L⁻¹d⁻¹) was recorded at zero and 25 Gy, respectively.

Gamma rays can generate free radicals (ROS), which have the ability to change the composition of cells in comparison with the slight penetration influence of UV-B. Hence, 60 Co-γ-rays were selected for irradiation due to their powerful penetration ability. In concomitant with the obtained results, Cheng et al. found that the lipid amount of Nitzschia sp. declined with increased irradiation dose (0-900 Gy). Agarwal et al. reported that the high irradiation doses extremely injure cell metabolism regulation complex and growth cease if cells lose their self-repair potential through injury recuperation. Considering that various strains had diverse irradiation vulnerability to nuclear irradiation, whereas under low dosages of γ-ray irradiation, some microalgal cells were still slightly damaged and recuperate their normal states within a brief period.
Figure 3. Growth curve of *D. splendida* cultured under different concentrations of nutrients and gamma radiation doses. (A) Nitrogen, (B) Phosphorous, (C) Magnesium, and (D) Carbonate, and (E) gamma radiation. Results represent mean ±SD of three replicates.
Figure 4. Lipid production of *D. splendida* cultured under different concentrations of nutrients and gamma radiation doses. (A) Nitrogen, (B) Phosphorous, (C) Magnesium, and (D) Carbonate, and (E) gamma radiation. Different small letters on the bars indicate significant difference at *p*<0.05. Results represent mean ±SD of three replicates.
Table 1. Kinetics of cell growth and lipid production of D. splendida under on different nutrients stress

<table>
<thead>
<tr>
<th>Nutrient concentration (mg L⁻¹)</th>
<th>Biomass productivity (BP) (mg L⁻¹ d⁻¹)</th>
<th>Maximum specific growth rate (µₘₐₓ) (d⁻¹)</th>
<th>Biomass yield (BY) (g L⁻¹)</th>
<th>Lipid productivity (LP) (mg L⁻¹ d⁻¹)</th>
<th>Lipid yield (LY) (g L⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>0</td>
<td>0.71 ± 0.004c</td>
<td>0.50 ± 0.02d</td>
<td>2.93 ± 0.16be</td>
<td>0.088 ± 0.003c</td>
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<td></td>
<td>380</td>
<td>17.65 ± 1.47c</td>
<td>55.54 ± 0.04e</td>
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<td>0.088 ± 0.005c</td>
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<td>750</td>
<td>25.93 ± 2.13e</td>
<td>60.60 ± 0.02c</td>
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<td>1500</td>
<td>31.73 ± 0.83b</td>
<td>90.80 ± 0.01b</td>
<td>5.37 ± 0.12a</td>
<td>0.152 ± 0.001a</td>
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<td></td>
<td>3000</td>
<td>42.06 ± 2.25b</td>
<td>99.99 ± 0.055b</td>
<td>1.15 ± 0.05a</td>
<td>5.58 ± 0.31a</td>
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<td>K₂HPO₄</td>
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<td>21.36 ± 1.20b</td>
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<td>40</td>
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<td>80</td>
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<td>160</td>
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<td>MgSO₄·7H₂O</td>
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<td>0.065 ± 0.001b</td>
<td>0.41 ± 0.02d</td>
<td>1.66 ± 0.11c</td>
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</table>

All cultures were incubated under continuous illumination of 40 µE m⁻² s⁻¹ and temperature of 25±1 °C with aeration rate of 1.25 L/min for 25 days. Different superscript letters within the same column indicate significant difference at p<0.05. Results represent mean ±SD of three replicates.

Table 2. Kinetics of cell growth and lipid production of D. splendida exposed to different gamma radiation doses

<table>
<thead>
<tr>
<th>Gamma radiation (Gy)</th>
<th>Biomass productivity (BP) (mg L⁻¹ d⁻¹)</th>
<th>Maximum specific growth rate (µₘₐₓ) (d⁻¹)</th>
<th>Biomass yield (BY) (g L⁻¹)</th>
<th>Lipid productivity (LP) (mg L⁻¹ d⁻¹)</th>
<th>Lipid yield (LY) (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>31.73 ± 0.83a</td>
<td>0.084 ± 0.004a</td>
<td>0.90 ± 0.01a</td>
<td>5.37 ± 0.12a</td>
<td>0.152 ± 0.001a</td>
</tr>
<tr>
<td>25</td>
<td>28.67 ± 1.28b</td>
<td>0.079 ± 0.002b</td>
<td>0.82 ± 0.04a</td>
<td>5.24 ± 0.43a</td>
<td>0.149 ± 0.012a</td>
</tr>
<tr>
<td>50</td>
<td>27.09 ± 1.04a</td>
<td>0.079 ± 0.002b</td>
<td>0.78 ± 0.03b</td>
<td>3.96 ± 0.26b</td>
<td>0.113 ± 0.006b</td>
</tr>
<tr>
<td>100</td>
<td>11.76 ± 1.05c</td>
<td>0.052 ± 0.006c</td>
<td>0.40 ± 0.02c</td>
<td>1.93 ± 0.19c</td>
<td>0.066 ± 0.003c</td>
</tr>
<tr>
<td>200</td>
<td>12.69 ± 0.25c</td>
<td>0.057 ± 0.001c</td>
<td>0.42 ± 0.01c</td>
<td>2.24 ± 0.02c</td>
<td>0.073 ± 0.007c</td>
</tr>
<tr>
<td>300</td>
<td>11.44 ± 0.14c</td>
<td>0.057 ± 0.002c</td>
<td>0.38 ± 0.01c</td>
<td>1.84 ± 0.11c</td>
<td>0.060 ± 0.003c</td>
</tr>
<tr>
<td>500</td>
<td>7.41 ± 1.27d</td>
<td>0.046 ± 0.006c</td>
<td>0.27 ± 0.03c</td>
<td>0.89 ± 0.18d</td>
<td>0.032 ± 0.005d</td>
</tr>
<tr>
<td>1000</td>
<td>4.53 ± 0.61e</td>
<td>0.032 ± 0.001e</td>
<td>0.21 ± 0.02e</td>
<td>0.53 ± 0.09d</td>
<td>0.024 ± 0.003d</td>
</tr>
</tbody>
</table>

All cultures were incubated under continuous illumination of 40 µE m⁻² s⁻¹ and temperature of 25±1 °C with aeration rate of 1.25 L/min for 25 days. Different superscript letters within the same column indicate significant difference at p<0.05. Results represent mean ±SD of three replicates.

Fatty acid composition

The fatty acid composition of D. splendida was given in Table 3. The FAME mainly contains saturated fatty acids (SFAs, 63.33 %) and unsaturated fatty acids (UFAs, 37.02%), also, the carbon chain lengths were from C12 to C24. Among the identified FAs, C16:0 was found to be present in higher concentration about 43.58 % followed by 19.22 % of C18:2, 10.72 % of C15:0 and 9.40 % of C16:1. The amounts of other FAs species were 17.08 % of the total FAs. Also, PUFAs and MUFA values recorded 22.8 % and 14.2 %, respectively.
Table 3. Lipid profile of *D. splendida* lipid cultivated on BG11 medium

<table>
<thead>
<tr>
<th>FA types</th>
<th>FA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric acid (C12:0)</td>
<td>1.15</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>3.10</td>
</tr>
<tr>
<td>Pentadecanoic acid (C15:0)</td>
<td>10.72</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>43.58</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)</td>
<td>9.40</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>0.64</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>4.80</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>19.22</td>
</tr>
<tr>
<td>Linolenic acid (C18:3)</td>
<td>3.60</td>
</tr>
<tr>
<td>Arachidic acid (C20:0)</td>
<td>0.46</td>
</tr>
<tr>
<td>Behenic acid (C22:0)</td>
<td>1.05</td>
</tr>
<tr>
<td>Lignoceric acid (C24:0)</td>
<td>2.28</td>
</tr>
<tr>
<td>Saturated fatty acids (SFAs)</td>
<td>63.33</td>
</tr>
<tr>
<td>Unsaturated fatty acids (UFAs)</td>
<td>37.02</td>
</tr>
<tr>
<td>Monounsaturated fatty acids (MUFAs)</td>
<td>14.2</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids (PUFAs)</td>
<td>22.82</td>
</tr>
</tbody>
</table>

FA, fatty acid

Regarding the biodiesel formation from *D. splendida*, the green microalgal lipid usually has a FA content of mostly C16 and C18 FAs that is akin to that of vegetable oils, and so appropriate for biodiesel formation. The C16-C18 FAs of *D. splendida* were 80.55%, which can give the best compromise between oxidative stability and cold flow properties. MUFAs, which mainly formed of C16:1 and C18:1, are regarded as the most preferable components for forming biodiesel, and they give the best compromise between oxidative stability and cold flow properties.

The tested microalga had distinctively higher amounts of C16 and C18 which were closer to those of *Haematococcus pluvialis* (76.6%) and *D. splendida* demonstrated considerable amount of C18:2 and C18:3, formed in low melting points, and are preferable for the improvement of the low temperature properties of biodiesel.

Conclusion:

The impact of media components, nutrients stress and γ- radiation on the biomass and lipid production of *D. splendida* was studied. The highest *BY* and *LY* were achieved when alga culturing on BG11 medium. The maximum *μ max* was obtained at high N, P and Mg as well as low CO2. While the highest *LC* was observed under nutrients limitation. Additionally, high γ-radiation doses expressed a negative influence on both growth and lipid production. The C16-C18 FAs of *D. splendida* were 80.55% which firmly manifested that *D. splendida* is a promising source for biodiesel formation.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Cairo University.

Authors' contributions statement:

Conception, Shanab SMM, Ali HEA; Design, acquisition of data and analysis, Ali HEA; Interpretation, Shanab SMM; drafting the MS, SMM, Ali HEA, Revision, and proofreading, Shanab SMM, Ali HEA, Abo-State MAM

References:


تأثير تركيب الاسطوان الغذائية والمغذيات وأشعة جاما على إنتاج الكتلة الحيوية والدهون من الطحلب الدقيق Dictyochloropsis splendida

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الخلاصة:
يعتمد إنتاج الديزل الحيوي من الطحالب الدقيقة على إنتاج الكتلة الحيوية ومحتوى الدهون. يتم التحكم في زيادة إنتاج الكتلة الحيوية BG11, BBM, Urea (media) وتركز الدهون بواسطة عدة عوامل. في هذا العمل تم دراسة تأثيرات ثلاثة أوساط غذائية خاصة برزاعة الطحالب Dictyochloropsis splendida وبعض المغذيات (الفيتامينات والنيتروجين والكربون) وأشعة جاما على نمو وانتاج الدهون للطحالب عندما الحصول على أعلى إنتاج للكتلة الحيوية والدهون لطحلب Dictyochloropsis splendida. تم زراعة الطحلب على الوسط الغذائي BG11 11 ميجرام / لتر نتروجين أو 160 ميجرام / لتر مغنيسيوم أو 3000 ميجرام / لتر فوسفور أو محتوى الدهون زاد. بينما عند غياب المغذيات فان تراكم الدهون زاد. في الظروف المعينة، كانت الدون المستخلصة من الطحلب تتكون من نسبة عالية من الاحماس الدهنية المشبعة (SFAs, 63.33%) والمحاسبة النقص في الدهون وحمض البيتا-كابويليك (palmitic) وحمض الستيريك (stearic) وحمض الغلوكليك (glucolic) وحمض البيتا-كابويليك (palmitic) وحمض الستيريك (stearic) وحمض الغلوكليك (glucolic)

الكلمات المفتاحية: وقود الديزل الحيوي، Dictyochloropsis splendida، عينات جاما، عناصر الغذائية.