Impact Of Different Nitrogen Concentrations On Biomass Productivity, Lipid Content And Target Fatty Acids Within Chlorella Sp. And Desmodesmus Quadricaudatus To Enhance Biodiesel Production

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Abstract: At constant time and temperature, pure cultures of Chlorella sp. and Desmodesmus quadriquadatus studied under different nitrate concentrations of 375, 186, 94, 47, 23 and 0.0 mgl^{-1} to enhance biodiesel production. Nitrate reduced gradually from concentration to another each 7-day cultures. Biomass for all cultures estimated as chlorophyll-a (μgl^{-1}) and dry cell weight (gl^{-1}). For all 7 days` cultures, biomass productivity ($mgl^{-1}d^{-1}$), lipid content (% biomass) and target fatty acids (%TFAs) for biodiesel production were detected. Because biodiesel quality and quantity affected by growth and lipid content; nitrate concentrations of 23 and 375 mgl^{-1} were suitable to cultivate Chlorella and D. quadricaudatus for production of biodiesel where Chlorella results` were; biomass productivity; 263$mgl^{-1}$, lipid content; 87.49%DCW and TFAs; 42.71% while at 375 $mgl^{-1}$ $NaNO_3$ D. quadricaudatus results` were; biomass productivity; 290.3mgl^{-1}, lipid content; 57.98%DCW and TFAs; 65.39%. Biodiesel samples produced from both species accepted for both ASTM and EN standards. In this paper, the highest percent in lipid content and biomass productivity for Chlorella sp. and Desmodesmus quadricaudatus comparing with other literature recorded. In addition, this paper considered the time as an agent for biodiesel production, that no other paper had considered.

Keywords: Biodiesel, Biomass productivity, Microalgae, Nitrate, Target Fatty Acids, **Abbreviations:** TFAs; Target Fatty Acids, CN; Cetane Number, SV; Saponification Value, DCW; Dry Cell Weight, CFPP; Cold filter plugging point, Gas Chromatography; GC.

1. INTRODUCTION

Due to oil shortage; alternatives as Renewable energies must emerge. There are three types of renewable energies; solar, wind and biomass energy. Biomass energy is the key for biodiesel production. Biodiesel is a fatty acid methyl or alkyl ester which derived from either vegetable oils or animal fats [1]. It is biodegradable and has no sulfur emissions [2]. There are many sources for biodiesel as plants like peanut and soybean [3] and microalgae or what known as oilgae[4]. Microalgae are microscopic organisms that uptake carbon dioxide for photosynthesis with highest efficiency. Using microalgal biomass for fuels conversion was first suggested in 1950s [5]. They are interesting sources for biodiesel production [6] due to their high oil content (1–85% of the dry weight) [7] and they don't compete on land or water.

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The key parameters that affect economic feasibility of algae for biodiesel production are biomass productivity, lipid cell content, and overall lipid productivity [8]. Chlorophyceae and Bacillariophyceae are the most reliable sources for biodiesel [5] because of their highly biomass productivity and oil content. Microalgae have oils as membrane components and storage materials. Under nitrogen or silica stress algae tend to stop growth and division to use all their energy for lipid synthesis [9]. Fatty acids are the precursor for biodiesel production. Microalgal cells have fatty acid patterns differ from plant cells. Unsaturated C18 fatty acids and elongation of carbon chains are the main differences between microalgae cells and plant oils [10]. Oleic acid within algal cells has a great significance as it provides a balance between the fuel properties of biodiesel [11]. Since biodiesel oxidize properties affected by its fatty acids composition, linoleic acid methyl ester has a very high oxidation instability, and can easily. Therefore, the linoleic acid content of algal oil, which evaluated for biodiesel production, should be in low amounts to prevent oxidation of the product and provide a better combustion [12]. It found that the cetane number of oleic acid methyl ester is higher than that of linoleic acid methyl ester [13]. The fatty acid profile of oil is very important for the cetane number [14]. Fatty acid profile of microalgae changed with changing culture conditions weather chemical or physical ones as nutrient starvation, salinity, and pH. Nitrogen depletion is the most investigated parameter for excessive lipid accumulation [15,16]. The composition of nutrient media (Nitrogen, carbon, phosphorus and trace metals) is one of the most significant factors that affect growth parameter and biochemical composition of microalgae [17, 18]. It is easy to induce oil content of 20–30% in several microalgal species [19]. Chlorella sp. and Desmodesmus quadricaudatus are belonging to Chlorophyceae. Chlorella sp. (M. Beijerinck) [20] is a unicellular oleaginous alga [21], has a potential application

in biodiesel production; its lipid content 20-50% [22]. Desmodesmus quadricaudatus (Turpin) Hegewald [23] is a colony that has a potential application for biodiesel production, although there is no pilot production [24]. Chlorella is preferred as a raw material in many studies because of its properties [16]. As a Chlorella species, Chlorella protothecoides shows great productivity of lipid production under various environmental conditions using different carbon sources. Fatty acid content of Chlorella protothecoides (C. protothecoides) oil is rich in oleic acid. [25]. In Chlorella vulgaris lipid contents of 40% and 56.6% (dcw) were recorded, when grown respectively in low nitrogen- and iron-supplemented medium [26, 27]. Scenedesmus obiliquus studied as a potent source for biodiesel under varied pH, temperature, and spectral quality, in presence of heavy metals, heat and chilling stresses, N and P deficiencies/ limitations to enhance lipid accumulation. The major factors affecting the performance of culture in the terms of lipid yield are the concentrations of nitrate, phosphate, and sodium thiosulphate. Time culture found to affect lipid productivity significantly [28]. Former studies indicated that Scenedesmus spp. was a potential microalgal species for lipid production, and the production was relative to incubation conditions, including CO2 supplement, nutrient condition, and temperature [29]. The aim of this paper is to study the effect of different nitrate concentrations (375, 186, 94, 47, 23 and 0.0 $mgL^{-1}NaNO_3$) on growth and lipid content of Chlorella sp. and Desmodesmus quadricaudatus towards biodiesel production. All species grown in BG-11 medium. Moreover, compare the results of studied species that used for biodiesel production in literature.

2. MATERIALS AND METHODS

2.1 Isolation, Purification and Identification of algal strains

Chlorella sp. and Desmodesmus quadricaudatus samples isolated, purified and identified as reported in [30]. Sterilized BG11 medium used in all steps, either in solid or liquid phase. BG-11 medium composed of (g l^{-1}), $NaNO_3$, 1.5; $K_2HPO_4 \cdot 3\ H_2O$, 0.04; $MgSO_4 \cdot 7\ H_2O$, 0.075; $CaCl_2 \cdot 2\ H_2O$, 0.036; citric acid, 0.006, ferric ammonium citrate, 0.006; Na_2EDTA, 0.001; Na_2CO_3, 0.02. In addition to 1 ml of trace metal solution (including H_3BO_3, 2.86 g; $MnCl_2 \cdot 4\ H_2O$, 1.81 g; $ZnSO_4 \cdot 7\ H_2O$, 0.222 g; $Na_2MoO_4 \cdot 2\ H_2O$, 0.390 g; $CuSO_4 \cdot 5\ H_2O$, 79 mg and Co $(NO_3)_2 \cdot 6\ H_2O$, 49.4 mg l^{-1})

2.2 Experimental design

To study the effect of nitrate limitation on growth and lipid content, 400 ml of Chlorella sp. and Desmodesmus quadricaudatus suspensions inoculated separately in sterilized 4l BG-11 medium with nitrate concentration 375mgl^{-1}. Nitrogen reduced to a half amount gradually each 7 days' cultures to finally had cultures cultivated in 375, 186, 94, 47, 23 and 0.0 mgl^{-1} $NaNO_3$. These cultures left to grow for 28 days and their biomass as chlorophyll-a and dry cell weight (DCW) estimated weekly. For day 7 cultures, biomass productivity, lipid content and fatty acid profiles investigated. In all cultures, 6l capacity bottles used. The bottles kept in 23±2ºC and 82.62 $\mu molm^{-2}sec^{-1}$. All cultures aerated by sterilized air using air pumps (Taoran T-03, China). All cultures replicated in three to calculate standard deviation and for more accuracy. To test biodiesel samples, 2 l of Chlorella sp. and D. quadricaudatus suspensions inoculated separately in sterilized 15 l BG-11 medium with nitrate concentration 375 mgl^{-1} and cultivated for 28 days. For these cultures, 18l capacity bottles used. The bottles kept in the same room temperature and continuous light as mentioned above.

2.3 Nitrate concentration determination

Nitrate concentration in the algal suspension determined according to [30]. A certain volume of sample (0.25 ml) mixed thoroughly with 0.8 ml of 5% (w/v) salicylic acid/H_2SO_4. After 20 minutes at room temperature, 19 ml of 2 N NaOH added to raise the pH value to 12 then samples cooled to room temperature and measured at 410 nm against 0.25 ml dis.H_2O with the normal reagents solution as a blank.

2.4 Measurement of algal growth

The changes of biomass measured weekly as chlorophyll-a (20ml) and dry cell weight (1 L) for Chlorella sp. and D. quadricaudatus. Chlorophyll-a determined according to [31] using spectrophotometer (6800 Double beam U.V./Visible Spectrophotometer, Jenway, made in England). Specific growth and the maximum growth rates calculated by (1); Specific growth rate; $\mu = \ln (N1–N2)/ (t1–t2)$ (1) Where N1 and N2 are biomass at time t1 and t2, respectively [32]. Dry cell weight of algal cells determined gravimetrically according to [33]. using a digital balance (Sartorius AG, BL-21OS, made in Germany).

2.5 Harvesting of algal biomass

The colonies of D. quadricaudatus were self-settled while Chlorella sp. did not [30]. To harvest the biomass in 15 l D. quadricaudatus, the culture left to precipitate then bottom layer of cells collected while for Chlorella sp. 1.8 m mol alum l^{-1} (Aluminum Sulphate octadecahydrate; $Al_2(SO_4)_3 \cdot 18\ H_2O$, molar mass 666.42 g mol^{-1}) added to the culture and mixed for 2 min thereafter left to settling. All collected biomass put in oven at 30°C for 2 days until dryness.

2.6 Biomass productivity and lipid contents

Volumetric biomass productivity (PBiomass) calculated as (2); PBiomass ($mgl^{-1}d^{-1}$) = $(X_2 - X_1) / (t_2 - t_1)$ (2) Where $X_1$and X_2 were the biomass dry weight concentrations (mgl^{-1}) on days t_1 (start point of cultivation) and t_2 (end of cultivation), respectively [8]. Lipid content calculated by the following equation; (3): Lipid Content (%) = wt. of lipid (g) × 100/ wt. of culture (g) (3) [34].

2.7 Extraction of alga lipid

A definite dry weight was poured all night in hexane: ether (1:1, V: V) to extract oil, this step was repeated until the extract became hyaline [35]. Then, according to [36] the extract evaporated in vacuum to release solvents using rotary evaporator (Diagonal Condenser-RE300, Vacuum 1 mm Hg, made in U.K.).

2.8 Fatty Acid Analysis

The extracted oil methylated according to [37].

2.9 Trans-esterification Process

Trans-esterification conducted with adding 24 ml sodium methoxide as a catalyst to extracted oil with molar ratio 1:135 stirred for 3 hours at 23±2ºC. The mixture was transferred into a separating funnel and left to settle for 16 hours. Top layer was the biodiesel layer while the bottom one had glycerin and other byproducts. Biodiesel layer transferred into another separating funnel and mixed vigorously with 5% water for washing, then left for settle [36]. Prepare 5% water by adding 5g 114 NaCL to 100ml dis.H2O.

2.9.1 Instrumental analyses; GC-MS analyses

Fatty acids and biodiesel analyzed using gas chromatography (Perkin Elmer Auto system XL) equipped with flame ionization detector (FID), fused silica capillary column DB-5 (60 m × 0.32 mm i.d.). The oven temperature maintained initially at 150 °C and programmed from 150 °C to 240 °C at rate 3 °C min^{-1}, then held at 240 °C for 30 min. The injector temperature was 230 °C. Detector temperature was 250 °C and carrier gas was Helium with flow rate of 1 ml min^{-1}).

2.10 Biodiesel quality

Physio-chemical characters of biodiesel samples investigated. Biodiesel samples were analyzed by gas chromatography (GC) to record their fatty acid methyl esters profiles. Acid value determined according to [38] where iodine value [39] measured for biodiesel samples. Cetane number (CN) was evaluated using [40] equation; (4); CN= 46.3 + (5458/SV) – (0.225 * IV) (4) Where SV is the saponification value and IV is the iodine value.

3. Results and Discussion

3.1 Effect of nitrate concentrations on microalgae growth and lipid accumulation

Chlorella sp. and D. quadricaudatus had high maximum specific growth rates of 3.02 and 2.96 d^{-1}, respectively [30]. The maximum growth rate is a significant factor for algal mass production [41]. [41] recorded a maximum specific growth rate (μ_{max}) for Scenedesmus spinosus (newly, Desmodesmus spinosus) of 3.0 d^{-1} which is close to the obtained data. [26] recorded a maximum growth rate for Chlorella vulgaris of 0.99 d^{-1}. According to [30] nitrogen was undetected in the culture suspension of Chlorella sp. and D. quadricaudatus on day 6 and day 12, respectively. The impact of certain nitrate concentrations of 375, 186, 94, 47, 23 and 0.0 mgL^{-1}NaNO3 on chlorophyll-a concentration (chl-a) and dry cell weight (DCW) detected weekly in cultures of Chlorella sp. and D. quadricaudatus. Chlorella sp. reached a maximum chl-a concentration of 3734 ± 84 μgl^{-1} at 47 mgl^{-1} $NaNO_3$ on day28. While D. quadricaudatus reached a maximum chl-a concentration of 1844 ± 20 μgl^{-1} on day 21 at 186 mgl^{-1} $NaNO_3$ (Fig. 1a,b). Chlorella sp. and D. quadricaudatus reached a maximum dry weight of 1.82 and 0.99 g l^{-1} on day 21 at 375 mg l^{-1} $NaNO_3$, respectively (Fig. 2a,b).

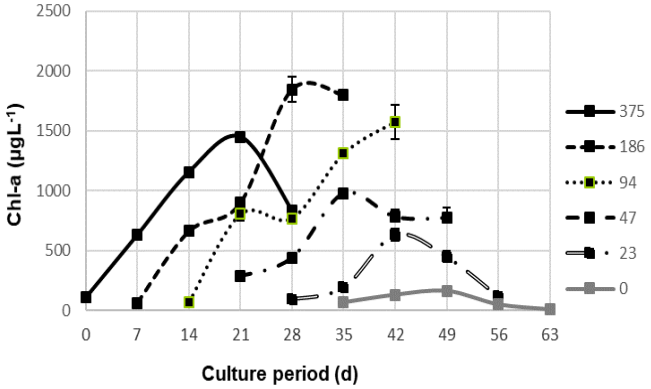

Fig. 1a. *Impact of different nitrate concentrations (mgl-1) on the growth measured as chlorophyll-a of Chlorella sp. measured weekly as Chlorophyll-a at 27±1°C, with continuous light. SD shows in plus and minus directions.*

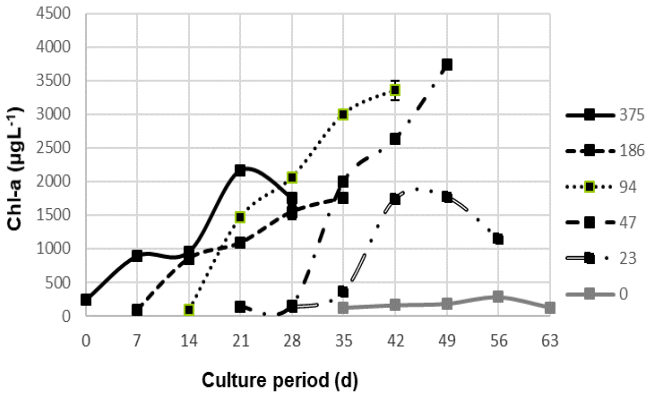

Fig. 1b. *Impact of different nitrate concentrations (mgl-1) on the growth measured as chlorophyll-a of D. quadricaudatus measured weekly as Chlorophyll-a at 27±1°C, with continuous light. SD shows in plus and minus directions.*

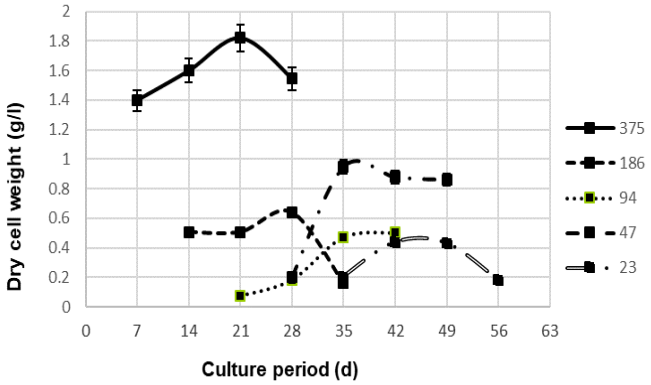

Fig. 2a. *Impact of different nitrate concentrations (mgl-1) on the growth measured as dry cell weight of Chlorella sp. measured weekly as Chlorophyll-a at 27±1°C, with continuous light. SD shows in plus and minus directions.*

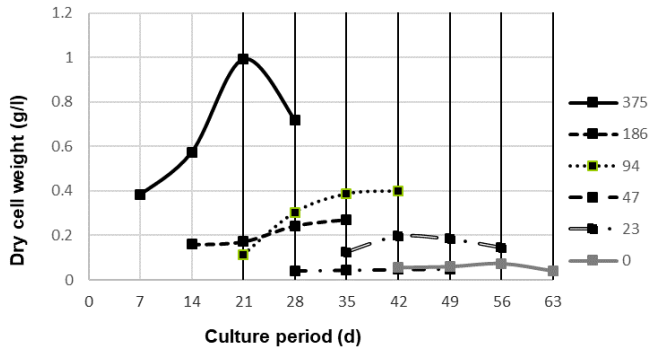

Fig. 2b. *Impact of different nitrate concentrations (mgl-1) on the growth measured as dry cell weight of D. quadricaudatus measured weekly as Chlorophyll-a at 27±1°C, with continuous light. SD shows in plus and minus directions.*

Biomass productivity ($mgl^{-1}d^{-1}$) and Lipid content (% DCW) in Chlorella sp. and D. quadricaudatus affected by different nitrate concentrations. The highest values of biomass productivity for Chlorella sp. and D. quadricaudatus were 752.3 and 290.3 $mgl^{-1}d^{-1}$ observed at 375 mgl^{-1} $NaNO_3$. The highest value of lipid content; 87.49% observed at 23 mgl^{-1} $NaNO_3$ for Chlorella sp. and 57.98% at 375 mgl^{-1} $NaNO_3$ for D. quadricaudatus, respectively (Table 1).

Table 1. *cell growth parameter and lipid content of Chlorella sp. and D. quadricaudatus cultured in different Nitrate concentrations for 7 days.*

Nitrate concentration (mgl^{-1})	*Chlorella* sp.			*D. quadricaudatus*		
	$X_{biomass}$ (mgl^{-1})	$P_{biomass}$ ($mgl^{-1}d^{-1}$)	$L_{content}$ (%DCW)	$X_{biomass}$ (mgl^{-1})	$P_{biomass}$ ($mgl^{-1}d^{-1}$)	$L_{content}$ (%DCW)
375	1397.9	752.3	2.56	382.5	290.8	57.98
186	507.3	288.4	60.49	159.7	64.3	5.68
94	75	27	39.76	113.5	92.7	34.14
47	201.9	263.3	8.33	38.4	18.5	51.47
23	203.6	246.1	87.49	123.7	70.8	8.92
0	162.2	15.8	24.52	54.6	27.9	19.79

[8] concluded that Chlorella sp. and Chlorella minutissima were fast-growing strains with the high biomass productivity. For Chlorella sp., biomass productivity was 451 to 495 mgl^{-1} d^{-1}. The lipid percentages of the Chlorella sp. and Desmodesmus sp. were between 15.66 to 21.02% [42]. [43] reported that when cultivated Chlorella and Desmodesmus for seven days into the BG-11 liquid medium their lipid content was 20% and 21%, respectively. According to [19], Chlorella has 28-32% oil dry wt^{-1}. [8] concluded that lipid content of Chlorella sp. was 18-30%. [44] reported that lipid content (% biomass) for Scenedesmus quadricauda and Chlorella sp. were 18.4 and 18.7, respectively.

3.2 **Effect of nitrate concentrations on target fatty acids for biodiesel within microalgae**

Biomass and fatty acids affected biodiesel quality and quantity. The choice of microalgae for biodiesel production needs a balance between species that grow quickly and produce oil in large quantities [19]. [45] stated that the saturated and unsaturated fatty acids as C16:0, C18:0, C18:1, C18:2 and C18:3 are common fatty acids used for biodiesel production. In present results, at different nitrate concentrations of 375, 186, 94, 47, 23 and 0.0 mgl^{-1}NaNO3, Chlorella sp. and D. quadricaudatus produced difference percent of significant fatty acids; TFAs; (C16:0, C18:0, C16:1, C18:1, C18:2, C18:3) for biodiesel production (Table 2).

Table 2. *Fatty acid profiles of autotrophically cultured algae Chlorella sp. and D. quadricaudatus cultured in different Nitrate concentrations (mgl-1) for 7 days.*

Fatty acids	*Chlorella* sp.						D. *quadricaudatus*					
	1	2	3	4	5	6	1	2	3	4	5	6
C14:0	1.11	26.24	17.42	24.12	3.1	15.71	3.26	4.86	18.24	19.93	6.57	18.39
C16:0	2.35	9.92	19.95	11.08	23.89	7.93	35.71	27.65	32.08	23.11	22.47	27.77
C18:0	1.11	1.36	2	4.97	6.59	3.39	4.88	NA	4.76	3.28	3.01	2.5
C18:1	NA	4.21	1.97	0.92	5.74	2.24	7.08	NA	2.38	3.28	31.31	16.99
C18:2	NA	0.2	2.78	NA	3.39	1.19	5.44	NA	2.02	NA	NA	1.22
C18:3	0.91	NA	NA	NA	NA	0.54	9.02	NA	NA	NA	NA	NA
C20:5	1.93	1.84	4.32	11.32	19.76	32.1	13.28	11.36	12.11	4	8.03	10.48
TFA[a]	5.48	41.93	44.12	41.09	42.71	31	65.39	32.51	59.48	49.6	3.36	66.87
SFA[b]	95.98	87.53	70.54	78.30	57.67	53.11	48.99	41.65	81.18	69.79	43.52	65.9
MUFA[c]	NA	4.21	1.97	0.92	5.74	2.24	7.08	NA	2.38	3.28	31.31	16.99
PUFA[d]	3.99	8.26	27.45	20.80	35.75	44.65	43.96	58.35	16.44	26.93	25.11	17.06

a Target fatty acids for biodiesel production (% of total fatty acids).
b saturated fatty acids (% of total fatty acids).
C monounsaturated fatty acids (% of total fatty acids).
d polyunsaturated fatty acids (% of total fatty acids).
1,2,3,4,5 and 6 related to different nitrate concentrations (375, 186, 94, 47, 23 and 0.0 mgl-1NaNO3) Chlorella sp. produced the highest concentration (44.12) of target fatty acids at 94 $mgl^{-1}NaNO_3$, while D. quadricaudatus produced the highest concentration (66.87) of target fatty acids at nitrogen free medium. According to these results, nitrogen limitation is the key for biodiesel production from Chlorella sp. and nitrogen starvation is the key for biodiesel production from D. quadricaudatus. [43] determined the fatty acid compositions of Chlorella and of Desmodesmus that cultivated for seven days into BG-11 liquid medium, showed similar fatty acid profiles C16:0, C16:4, C18:1, C18:2, and C18:3 as major components. Fatty acid profiles of Desmodesmus sp. show C16:0, C18:0, C20:0, C 16:1, C16:2, C16:3, C16:4, C18:1, C18:2, C18:3, C20:1 [46].

3.3 **Effect of nitrate concentration on biodiesel quality**
Chlorella sp. and D. quadricaudatus cultivated for 28 days in BG11 medium with 375 mgl^{-1} $NaNO_3$. The extracted oil converted into biodiesel via Transesterification process. Chemical composition and some physicochemical parameters determined for biodiesel samples. Biodiesel produced from Chlorella sp. was rich in linolenic acid methyl ester while Biodiesel from of D. quadricaudatus was rich in palmitic acid methyl ester (Table 3a).

Table 3a. *fatty acid methyl esters profiles for Chlorella and D. quadricaudatus grown in 375 mgl-1 NaNO3 for 28 days.*

Systemic names of Fatty acid methyl ester	Carbon number	%wt. *Chlorella* sp.	%wt. *D. quadricaudatus*
Tridecanoic acid	C13:0	**NA**	1.9
Myristic acid	C14:0	**NA**	1.02
Pentadecanoic acid	C15:0	**NA**	1.5
Palmitic acid	C16:0	6.12	62.5
Heptadecanoic acid	C17:0	3.19	3.5
Stearic acid	C18:0	27.03	4.6
Palmitoleic acid	C16:1	**NA**	0.35
Oleic acid	C18:1	7.57	2.9
Linoleic acid	C18:2	**NA**	9.5
Linolenic acid	C18:3	35.73	6.6
Eicosapentaenoic acid	C20:5	2.09	0.37
Arachidonic acid	C20:4	**NA**	2.97
Docsahexanaenoic acid	C22:6	**NA**	3.02

The physicochemical parameters include acid value, iodine value, cetane number, cold filter plugging point and high

heating value for biodiesel samples of Chlorella sp. and D. quadricaudatus investigated (Table 3b). The qualities of biodiesel samples produced from Chlorella and D. quadricaudatus are in range of ASTM and EN standers. Biodiesel samples produced from Chlorella and D. quadricaudatus have cetane numbers of 85.53 and 49.61 wt.%, respectively, and these results accepted with ASTM (> 47 wt.%). In addition, have iodine values of 68.645 and 100.05 gI 100g-11 for Chlorella and D. quadricaudatus respectively. The acid number for biodiesel is an indicator of free fatty acids [47]. Biodiesel samples produced from Chlorella and D. quadricaudatus have acid number of 62.655 ± 2.6 and 57.435 ± 1.9 mg $KOHg^{-1}$oil, respectively. Accordingly, these samples need some improvements.

Table 3b. *properties of biodiesel from Chlorella and D. quadricaudatus grown in 375 mgl-1 NaNO3 for 28 days.*

Property	Unit	Mean values In case of *Chlorella* sp.	Mean values In case of *D. quadricaudatus*	ASTM 6751 - 02 for Biodiesel (B100)	EN 14214 for Biodiesel (B100)
Acid Value	mg $KOHg^{-1}$ oil	62.655 (±2.058)	57.435 (±1.88)	0.5 max.	0.5
Iodine value	mg I_2 g^{-1}	68.645 (±2.623)	100.05 (±4.19)		120
Cetane number		58.53	49.61	47 minima	51
Cold filter plugging point	ºC	27.91	10.38		
High heating value	$Btu.lb^{-1}$	18645	18181	538581.76	

Cold filter plugging point (CFPP), indicates the flow performance of biodiesel at low temperature, was approximately 15ºC for the algal sample. Biodiesel samples produced from Chlorella and D. quadricaudatus have CFPP of 27.91 and 10.38ºC, respectively. Biodiesel samples produced from Chlorella and D. quadricaudatus have high heating value of 18645 and 18181 $Btu.lb^{-1}$, respectively.

4. Conclusion

In present study, we demonstrated the feasibility for high cell density and lipid content of Chlorella and D. quadricaudatus batch cultures. Different nitrate concentrations (375, 186, 94, 47, 23 and 0.0 mgL^{-1}NaNO3) were tested to enhance biodiesel production. From results we concluded that nitrate limitation is the key for biodiesel production from Chlorella sp. and nitrogen starvation is the key for biodiesel production from D. quadricaudatus. The lipid produced by Chlorella and D. quadricaudatus were model feedstocks for biodiesel production because of their high content of neutral lipids and appropriate fatty acid composition.

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