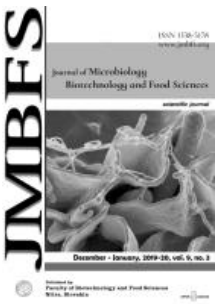

JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

COST EFFECTIVE CULTIVATION AND BIOMASS PRODUCTION OF GREEN MICROALGA *DESMODESMUS SUBSPICATUS* MB. 23 IN NPK FERTILIZER MEDIUM

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doi: 10.15414/jmbfs.2019/20.9.3.599-604

ARTICLE INFO

Received 4. 7. 2018
Revised 10. 6. 2019
Accepted 10. 6. 2019
Published 1. 12. 2019

Regular article

OPEN ACCESS

ABSTRACT

Microalgal biomass has emerged as a promising alternative to replace plant-based biofuel feedstock due to its higher lipid productivity. But cultivation of microalgae in expensive analytical grade culture media is a major obstacle in feasible algal biofuel production. Hence the present investigation was carried out to find an alternative, low-cost culture medium for the increased biomass yield and biochemical production of microalga, *Desmodesmus subspicatus* MB. 23. The strain was cultivated in different concentrations of 19: 19: 19 NPK media and checked for the algal biomass production and biochemical accumulation. Maximum algal cell density (5290 $\times 10^4$ cells/ ml), biomass yield and productivity (2.72 g/L, 60.87 mg/L/d) was attained in 2 g/ L NPK fertilizer medium, whereas 1g/L medium exhibited increased chlorophyll production (chl-a, 4.7 mg/g d wt, chl-b 1.7 mg/g d wt). High level of carotenoid accumulation (4.6 mg/g d wt) as well as lipid accumulation (29.5%) was found in 0.5g/L fertilizer media and Fatty acid methyl ester analysis of the strain showed abundant production of C16 and C18 fatty acids. Findings of the current study proved that, lower concentration of 19:19:19 NPK fertilizer can be used for the enhanced production of green microalgae. Other than cost effective biofuel production, NPK fertilizer grown *D. subspicatus* can also be used for the production of a wide range of metabolites such as food and feed additives, pharmaceuticals and cosmetics.

Keywords: Biofuel, Biomass production, *Desmodesmus subspicatus*, Carotenoids, NPK fertilizer

INTRODUCTION

Problems related to global shortage of energy sources can be handled by biofuel production through microalgal biomass cultivation. Due to high lipid productivity and lower land occupancy, the production of algal biofuel as a renewable source of energy has gained much interest in recent years (**Yin *et al*., 2012; Ji *et al*., 2015**). However, some of the practical difficulties in cultivation methodologies, harvesting technologies, extraction of algal lipid and transesterification processes are associated with the economical processing of microalgal oil (**Kumar *et al*., 2015**). As nutrient supplementation affects algal biomass production and lipid productivity, the operating cost associated with nutrient consumption is also one of the major obstacles in feasible algal biofuel production (**Caprio *et al*., 2015; Nayak *et al*., 2016**). Above all preparation of media and stock solutions are the most expensive as well as tedious part of algal culture. Therefore, utilization of readily available media can be followed to reduce the cost of algal biomass production (**Abu Hajar *et al*., 2017; Mangaiyarkarasi *et al*., 2017**). In that context, commercial fertilizers can be used as a nutrient source for cultivation and economically viable production of microalgae (**Xiney, 2016**).

Numerous studies were carried out for the biomass production of microalgae using commercial NPK fertilizers. Researchers have reported that the algal production in NPK fertilizers are cost- effective because of wide availability, solubility, well-defined nutrient composition and a similar to or a greater algal growth than commercial medium (**Sipauba-Tavares *et al*., 2017**). Majority of the studies were focused on the commercial production of *Chlorella* and *Spirulina* in NPK 16:4:6 (**Ashraf *et al*., 2011**), NPK 10:26:6 (**Kumari *et al*., 2015),** NPK 20:20:20+TE N: P: K (**Ammar, 2016**; **Mahmood *et al*., 2017**). A few studies have also optimized the biomass production of *Scenedesmus* sp. in fertilizer-based medium (**Nayak *et al*., 2016; Mangaiyarkarasi *et al*., 2017**). But no studies were reported on the commercial cultivation of *Desmodesmus subspicatus* in a fertilizer media.

D. subspicatus (previously known as *Scenedesmus subspicatus*) is one of the most widely used microalgae in research studies for industrial application and bioremediation purposes (**Grabski and Tukaj, 2008; Tukaj and Tukaj, 2010; Bascik-Remisiewicz *et al*., 2011; Pokora *et al*., 2011; Grabski *et al*., 2016; Toress *et al*., 2018**). The alga contains good amount of carbohydrates, proteins, lipid and pigments, and reports suggest the strain as a suitable candidate for biohydrogen production (**Dean *et al*., 2010; Chen *et al*., 2016; Eze *et al*., 2017; Correa *et al*., 2018**). A very recent study reported that the strain exhibits potent antioxidant properties and antibacterial activities (**Dantas *et al*., 2019**). *Desmodesmus subspicatus* has also been suggested as a promising algal feedstock for biodiesel production because of its high lipid content, productivity and high total fatty acid content, also the physical properties of the fatty acid methyl esters meet the required biodiesel quality standards (**Maazouzi *et al*., 2012; Gressler *et al*., 2014; Eze *et al*., 2017; Chaudhary *et al*., 2017; Ogbonna *et al*., 2018**).

So, the present study was focused on the cultivation of a green microalga, *Desmodesmus subspicatus* MB. 23, in a readily available commercial NPK 19:19:19 fertilizer medium to investigate the biomass production and lipid productivity. NPK 19:19:19 is a water-soluble fertilizer contributing nitrogen, phosphorus and potassium in equal proportion (19%). It contains balanced nutrients to improve vegetative and reproductive activities in the plant system. As it is useful for all crops, it is popular in Kerala, India as a foliar spray and the fertilizer is used in many research activities for the enhancement of crop production (**Das and Jana, 2015; Sudeep et al., 2018**).

MATERIAL AND METHODS

Microalga strain and culture conditions

The green microalga, *Desmodesmus subspicatus* MB. 23 (NCBI Accession number- KX235325) isolated from Cochin estuary was used for the study. The culture was maintained in the Marine Botany laboratory, Cochin University of Science and Technology, India; in 1 L Erlenmeyer flasks containing 500 mL modified Bold Basal Medium (BBM) at room temperature (28–30°C) with a light: dark period of 12:12 h and a light intensity of 36µmol photons/m^2/s and were shaken at regular intervals.

Experimental design

Suitable amount of NPK fertilizer (obtained from Krishi Vigyan Kendra, Ernakulam, Kerala, India.) was weighed and dissolved in double distilled water

to give 0.5%, 1%, 2%, 4%, 6%, 8% and 10% solutions and was diluted with fresh water to obtain 100 ml different concentrations of fertilizer media. All cultivations were done in 250 ml Erlenmeyer flasks that were incubated on an orbital shaker (120 rpm) at room temperature. Illumination was provided by white fluorescent lamp with a light intensity of 39µmol photons m^{-2} s^{-1} with a light and dark cycle of 12: 12 hours. 10% inoculum (10 ml) at late logarithmic phase, previously grown in NPK fertilizer medium was added to 100 ml media. Growth and biomass were compared by keeping regular BBM as a control.

Growth performance

Growth was calculated by the estimation of optical density (OD_{680}) using a UV-Vis spectrophotometer. Regression equations in terms of OD_{680} versus cell density and dry weight were calculated and used for the estimation of growth. The correlation established is given below:

Cell density $= O.D\ 680\ /0.003$ ($R^2 = 0.9994$) (1) Biomass yield $= O.D\ 680 \times 0.007 - 0.0014$ ($R^2 = 0.9832$) (2)

Biomass productivity (mg L^{-1} d^{-1}) was calculated using the following equation of **Ma *et al*., (2017)**, where b_2 and b are the biomass concentrations at the time t_2 and t. t_2 is the cultivation period of 45 days.

Biomass Productivity $= (b2 - b)/(t2 - t)$ (3)

Carbon dioxide fixation efficiency and pH changes

CO_2 consumed by the microalgae or the CO_2 fixation efficiency (mg L^{-1} d^{-1}) was calculated using the following equation **(Ho *et al*., 2010; Nayak *et al*., 2016)**: **B**

CO_2 fixation efficiency $= 1.88 \times$ Biomass productivity (4)

To study the changes in pH during cultivation, the media were checked by a Portable pH meter (Perkin Elmer, accuracy± 0.01).

Microscopy

Morphological observation of algal culture and cell counting during different growth phases were conducted by phase contrast microscope (Nikon, Eclipse E 200). The structural changes in *Desmodesmus subspicatus* MB. 23 were studied by scanning electron microscopy (SEM). Samples fixed with 2.5% glutaraldehyde followed by dehydration in ethanol were used for SEM and the images were taken at Sophisticated Test and Instrumentation Centre (STIC), Cochin university of science and technology, India.

Pigment composition

The determination of pigments, chlorophylls and carotenoids was conducted. For that, 1 ml of algal cultures were extracted with 10 ml of 90% acetone. To estimate chlorophylls incubation was done for 24 hours and for the successful extraction of carotenoid pigments an incubation period of 48 hours was standardized. The estimations were done as per the equations of **Strickland and Parson (1972).**

Determination of protein and lipid

Algal protein content was estimated by micro Bradford assay (**Bradford, 1976**). 800 µL of each of the sample solutions were treated with 200 µL of Bradford reagent. After vortexing, samples were incubated at room temperature for 15minutes and the absorbance measured at 595 nm. For the analysis of total lipids, 15 ml cultures were extracted with 15 ml chloroform/methanol solution (2/1, v/v) and were quantified gravimetrically using Folch's extraction technique (**Folch *et al*., 1957**). Neutral lipids were checked through Nile Red staining (**Damiani *et al*., 2010**). To improve the staining efficiency the concentration of stain was increased (500 µl Nile Red added to equal volume of algal culture) and the staining time was set to forty minutes. Lipid yield (%) was calculated as:

Yield (%) $= \frac{\text{Weight of the total lipid}}{\text{Weight of dry algal biomass}} \times 100$ (5)

Lipid productivity(mg /L/ d) $=$ Lipid concentration/ t (6)

Fatty acid analysis

Lipid fraction was derivatized by the method of **Cavonius *et al*. (2014)**. Analysis was carried out using 0.5 N methanolic sodium hydroxide followed by methylation with 14% BF_3 /methanol at 100°C for 5 min. Shimadzu Gas Chromatograph GC-2010 Plus connected with Stabilwax capillary column (30 m×, 0.25mm × 0.25µm) was used for FA profiling. All the experiments were conducted in triplicate, and data presented in mean values with standard deviation (SD) of three independent replicates. Statistical analysis was performed using SPSS 20.0 and a significant difference was considered at the level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of NPK fertilizer media on *D. subspicatus* growth

Different media with a varying nutrient composition significantly affects the cell biomass production and the production of various bio-chemicals (**George *et al*., 2014; Mahmood *et al*., 2017**). In the current study, the growth of green alga *Desmodesmus subspicatus* –a suitable candidate for biofuel production- was checked in different concentrations of NPK against BBM as a control for 45 days of experimental period. The growth performance of *D. subspicatus* MB. 23 revealed that the cell growth was significantly varied ($p \leq 0.05$) in NPK media with that of control. There was an accelerated growth of microalga in lower concentrations of fertilizer media when compared to Bold Basal Media.

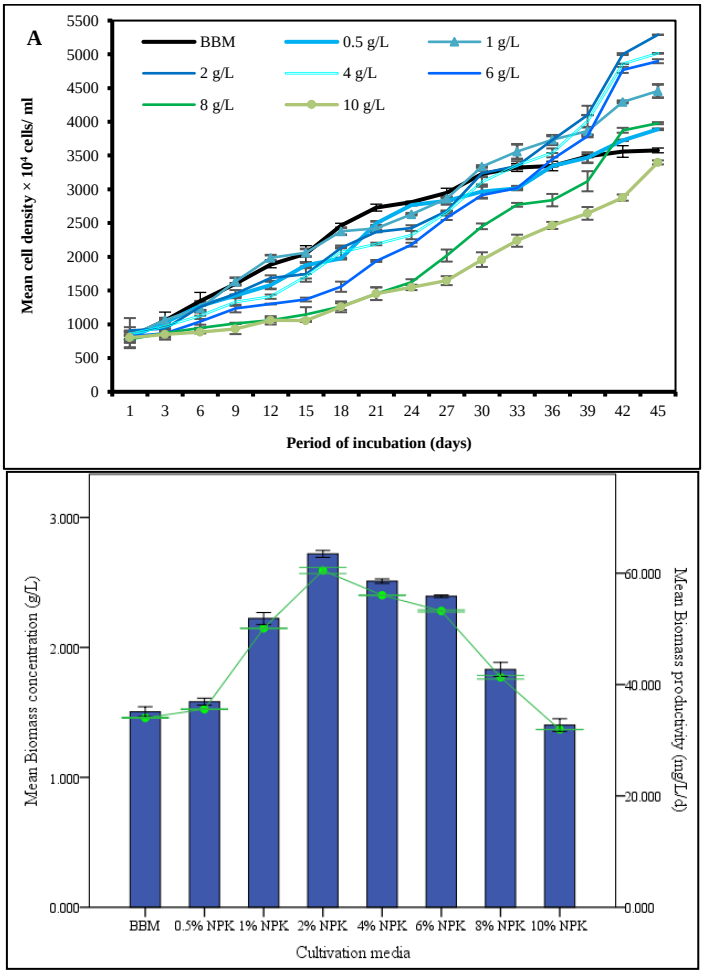


Figure 1 Growth of *D. subspicatus* MB. 23E in different concentrations of fertilizer media compared to BBM. (A) Cell density (B) biomass yield and biomass productivity

Maximum cell density was obtained in 2g/L fertilizer media (5290 ×10^4 cells/ ml) followed by 1g/L (4490.9×10^4 cells/ ml). The cell density observed in the control BBM medium was 3576.50× 10^4 cells/ ml (Figure 1 A). Both 1% and 2% NPK media were similar in terms of mean cell density. It should be noted that the growth phase was comparable in BBM and 0.5% fertilizer medium, while alga in higher concentrations of fertilizer media exhibited a prolonged lag phase. **Sipauba-Tavares *et al*., (2017)** reported to attain a maximum cell density of 25.5 × 10^6 cells/ ml when *Ankistrodesmus gracilis* was cultivated in 20:5:20 NPK fertilizer medium.

The potentiality of algae as bio-energy feed stock was determined by lipid production along with the amount of producible biomass (**Mandotra *et al*., 2014**). A yield of 2.72 g/L dry cell weight was obtained in 2% NPK concentration with a biomass productivity of 60.87 mg/L/d (Figure 1 B). The biomass productivity of 50.11 mg/L/d was similar and the biomass yield (2.2 g/L) obtained in the current study was higher in 1% fertilizer media, when compared to those by **Nayak *et al*., (2016)** in cultures of *Scenedesmus* sp. cultivated in NPK (10:26:26) fertilizer media. According to the authors, higher biomass yield of 0.9g/L was obtained in 1g/L fertilizer medium and a higher NPK concentration was found to lower the biomass yield. The study was conducted for 18 days and the microalga attained a stationary phase, whereas in the current study the cultivation extended up to 45 days to evaluate cultures in stationary phase and the difference in the results might be due to the variation in the percentage of nitrogen present in the two NPK fertilizer media. **Ammar (2016)** also reported a delayed stationary phase of growth in *Chlorella vulgaris* when cultivated in 80mg/L of 20:20:20 NPK concentration. **Gressler *et al*, 2014** yielded a density of 42.48 ×10^6 cells/ ml and a dry weight of 1277.44 mg/L for the strain *D. subspicatus*, when cultivated in waste water medium with CO_2 supply. This result

is significantly lower than the present study, which validates the enhanced biomass production in NPK fertilizer medium.

Effect of fertilizer media on morphological and physio- biochemical characteristics of *D. subspicatus* MB.23

The biomass of *Desmodesmus* sp. accumulates pigments and the accumulation of chlorophyll-a, b and carotenoids, act as factors to check the productivity of algal cultures (**Cheban *et al*., 2015**). Lower concentrations of fertilizer media were found to support accumulation of pigments. Chl-a (4.7 mg/g d wt) and chl-b (1.7 mg/g d wt) concentrations were highest in 1% fertilizer media whereas, carotenoid accumulation (4.6 mg/g d wt) was found in 0.5%. (Table 1) This result is contradictory to the reports of **Dean *et al*. (2010),** where *S. subspicatus* exhibited a reduction in the chlorophyll content when nitrogen concentration is lowered. In the current study maximal content of pigments were obtained in the stationary phase. However, the values obtained in the present study were lower than reported by **Cheban *et al*. (2015)** when *Desmodesmus armatus* was cultivated in RAS wastewater (11.17 Chl-a, 7.07 Chl-b, 12.05 carotenoides mg/g/d wt.). **Eze *et al*. 2017** also reported an accumulation of carotenoids in *D. subspicatus*.

Algal protein content is dependent upon nitrogen concentrations, and potassium is essential for several enzymes involved in protein synthesis (**Miriam *et al*., 2017**). *D. subspicatus* grown in NPK fertilizer medium did not show significant difference (p>0.05) in the total protein production among various concentrations. Maximum protein production of 140 mg/ L was found in 6% fertilizer media, while in BBM it was 114.8 mg/L. Comparatively a lower yield of protein (8.5%) per dry weight was obtained in the current study (Table 2) when compared to that observed (12.3%) in the same alga cultivated in fish silage (**Abdulsamad and Varghese, 2017**).

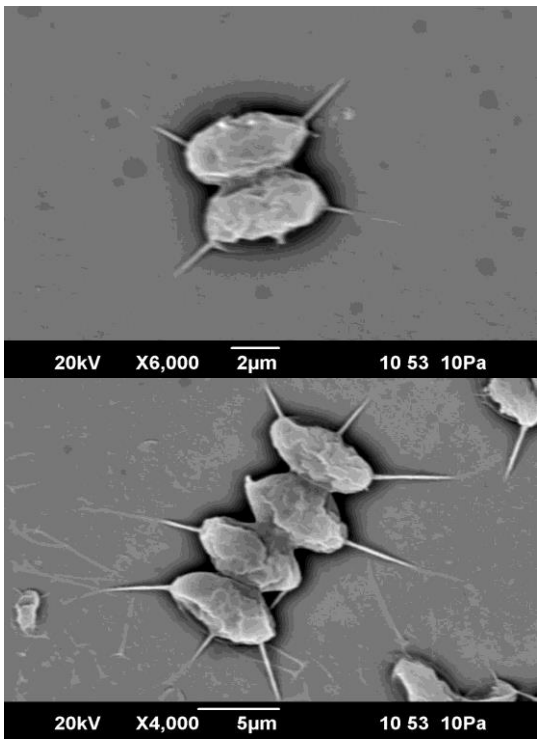
The changes in nutrient availability rapidly alters the metabolic responses of *D. subspicatus*. It was reported that high nitrogen treatments reduce the cell size, while in low or intermediate nitrogen the strain exhibits an increase in cell size (**Dean *et al*., 2010**). Genus *Scenedesmus* is well known for its phenotypic plasticity. **Pancha *et al*. 2014** reported that high concentration of nitrate and phosphate in growth medium makes most of the lab grown *Scenedesmus* to be unicellular in nature. An attempt was made to observe the morphological responses of *D. subspicatus* in different fertilizer media in comparison to control BBM.

Alga in fertilizer media was found to exhibit unicellular nature in initial stages of growth, while in control medium they were in four celled coenobia. Scanning Electron Microscopic studies of *Desmodesmus subspicatus* MB. 23 revealed the pectic cell wall layer and the morphological changes in spine production due to nutritional stress. In the initial stages where the nutrients were maximum, alga appeared as single-celled or two celled with spines originating from the four corners of the coenobium and as it reached the stationary phase, four celled coenobia were found with additional long spines of narrow endings. While the alga reached the decline stage, due to maximum nutrient stress there was an increase in the number of spines and it was found that in the dividing stages (auto spore formation) the spines were shedding and the broken spines were clearly visible in the SEM image (Figure 2 A- D). The findings matched with the results obtained in *S. acutus* when cultured under varying nutrient conditions (**Sterner *et al*., 1993**).

Table 1 Pigment composition of D. subspicatus MB. 23 on different NPK concentrations

Pigment composition (mg/G)	BBM	0.5% NPK	1% NPK	2% NPK	4% NPK	6% NPK	8% NPK	10% NPK
Chlorophyll -a	3.4± 0.09	4.2± 0.11	4.7± 0.11	3.4± 0.19	2.0± 0.26	1.9± 0.09	1.8± 0.10	1.2± 0.03
Chlorophyll -b	0.7± 0.03	0.8± 0.03	1.7± 0.05	0.8± 0.04	0.6± 0.04	0.3± 0.05	0.3± 0.02	0.1± 0.02
Chlorophyll a+ b	4.1± 0.14	4.9± 0.23	6.4± 0.06	4.1± 0.14	2.6± 0.20	2.2± 0.04	2.1± 0.07	1.3± 0.02
Carotenoids	2.5± 0.14	4.7± 0.11	3.8± 0.08	1.9± 0.13	1.9± 0.01	1.7± 0.06	1.5± 0.06	1.3± 0.08
Carotenoids/ Total chlorophyll	0.6± 0.08	0.9± 0.43	0.5± 0.01	0.5± 0.12	0.7± 0.04	0.8± 0.02	0.7± 0.01	1.1± 0.02

It was observed that in lower fertilizer concentrations, increased cell density and nitrogen starvation leads to the accumulation of underutilized nutrients, in turns affects physiological stress in the algae, which causes sedimentation with some adhesion complexes. As a result of this physiological stress, *D. subspicatus* lost its original shape, became fuzzy with shredded spines and accumulated more oil droplets. Therefore, a high biomass increase with good lipid accumulation can be observed in the lower concentration of fertilizer media (Figure 3A) and an increase in biomass with high lipid content will improve the efficiency of oil extraction and the downstream processing cost can be decreased (**George *et al*., 2014**).



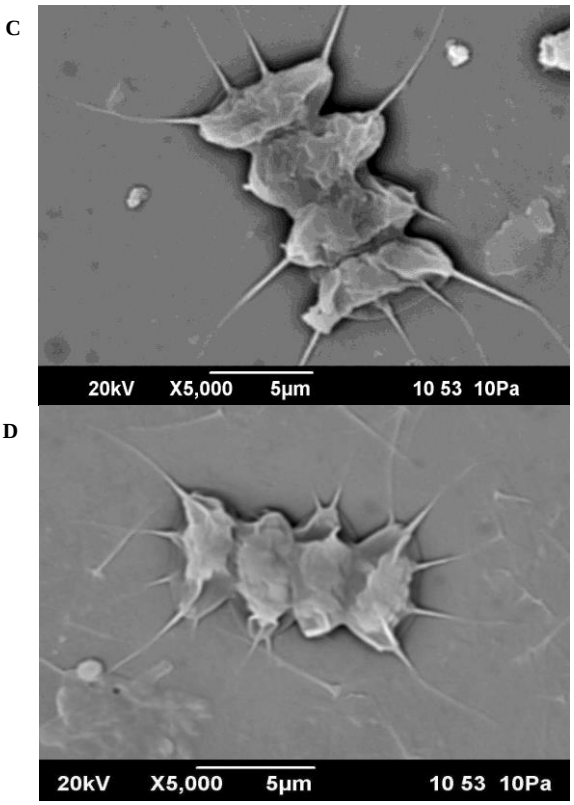
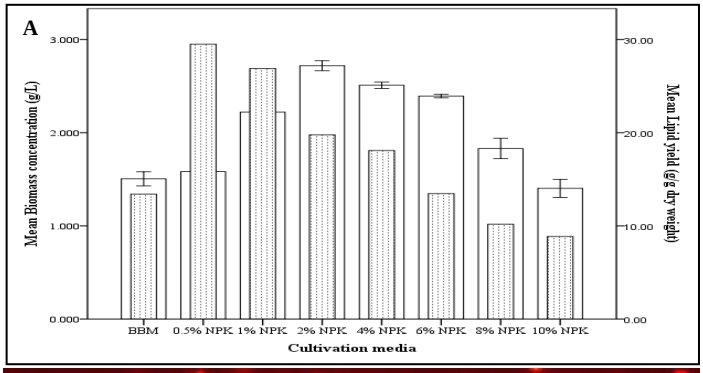


Figure 2 SEM images of *D. subspicatus* MB. 23. (A) alga in active log phase, (B) in early stationary, (C) in late stationary, (D) in decline phase.

Neutral lipids staining through Nile red method, showed that *D. subspicatus* cells grown in 0.5% fertilizer medium accumulated a higher ratio of lipid droplets (Figure 3B). The quantitative determination also supported this result that higher lipid yield (29.50%) and productivity (10.84 mg/L/d) attained in 0.5% fertilizer media. Comparatively, good lipid yield was obtained in 1% NPK concentration and the results were given in Table 2.



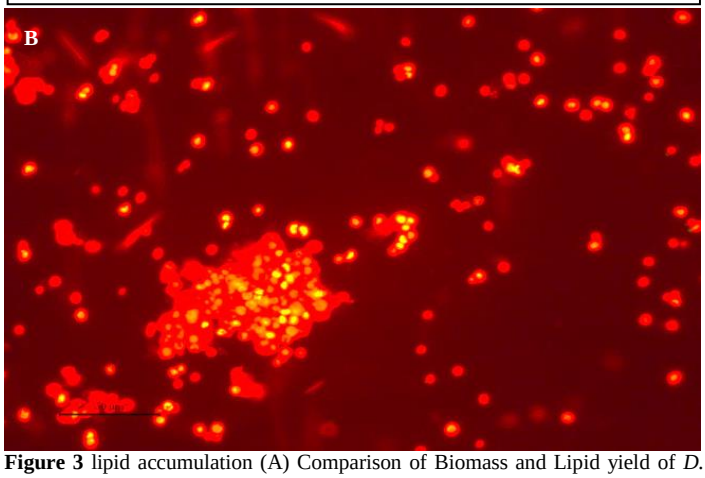


Figure 3 lipid accumulation (A) Comparison of Biomass and Lipid yield of *D. subspicatus* MB. 23 cultivated in different NPK concentrations and BBM (B) Nile Red staining image of *D. subspicatus* MB. 23 grown in 0.5 g/L NPK solution showing orange yellow stained oil droplets and with shredded spines.

Lipid yield obtained was similar to the previous work on the same species while cultivated in fish silage (**Abdulsamad and Varghese, 2017**). Highest lipid productivity for *D. subspicatus* (0.217g/L/d) was recorded when cultivated in a photobioreactor with a reflective broth guide (**Eze *et al.*, 2017).** A lipid yield of 28.44 % in NPK fertilizer medium was reported by **Nayak *et al.* (2016)** with a higher productivity of 18.87 mg/L/d for *Scenedesmus*. As the lipid productivity directly correlates with harvesting time, the increased value in the study is due to a shorter cultivation period. **Chaudhary *et al.* (2017**) attained a higher lipid yield (29%) of *D. subspicatus* under nitrogen starvation, perhaps the current study agrees with the report of **Welter *et al.* (2013)** that a higher biomass concentration can increase lipid concentration, as an initial high nitrogen will eventually deplete and lead to an increase in the lipid content, but needed a long cultivation period.

CO_2 consumption rate and pH changes in different NPK concentrations

The rate of CO_2 fixation is directly related to the growth of photosynthetic microorganism (**De-Morais and Costa, 2007**) and the CO_2 consumption rate is related to biomass productivity. In the present study, maximum rate was of 112.43 mg/L/d obtained in 2% fertilizer medium (Figure 4). The rate was comparatively higher than that reported by **Nayak *et al.* (2016)** (94mg/L/d) and lower (390.2 mg/L/d) than that reported by **Ho *et al.* (2010)** in *Scenedesmus obliquus*. Changes in pH was significant, as it showed an increase from an acidic pH (ranged from 5.2 to 5.6) to a basic final pH (7.7 to 8). Since an elevated pH can enhance ammonia and phosphorous removal, it was obvious that NPK fertilizer media was consumed by the microalga with effective reduction in these components. **Jena *et al.* (2012)** reported that an increase in final pH is due to the consumption of CO_2 leading to OH^- ion accumulation and the present study agrees with this finding, as a high CO_2 consumption rate was obtained with a final raised pH level. Similar elevated final pH was also obtained in the studies of **Gressler *et al.* (2014)** and **Chaudhary *et al.* (2017**) for *D. subspicatus*.

Table 2 Biomass and lipid productivity of *D. subspicatus* MB. 23 grown on different NPK concentrations.

Cultivation medium	Biomass (g/L)	Biomass productivity (mg/L/d)	Protein yi (%)
BBM	1.50± 0.04	34.04± 0.04	5.8±0.26
0.5 g/L	1.58± 0.02	35.58± 0.02	6.5±0.53
1 g/L	2.22± 0.05	50.11± 0.05	6.8±0.19
2 g/L	2.72± 0.02	60.86± 0	7.2±0.91
4 g/L	2.51± 0.01	56.05± 0.01	7.7± 0.53
6 g/L	2.39± 0.05	53.35± 0.05	8.5± 0.75
8 g/L	1.83± 0.05	41.53± 0.05	7.9± 0.25
10 g/L	1.40± 0.04	31.93± 0.04	7.8± 0.69

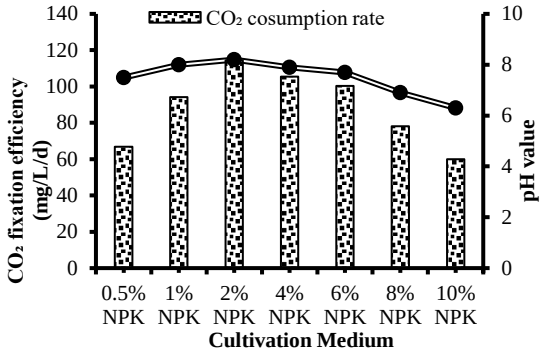


Figure 4 CO_2 fixation efficiency and final pH value of *D. subspicatus* MB. 23 in different concentrations of fertilizer media

Fatty acid composition of *D. subspicatus*

The fatty acid profile of *D. subspicatus* in the late stationary phase was detected to check the biofuel producing efficiency. The species had C16:0 (35.5%), C18:1 (22.3%), C18:2 (15.6%) and C18:3 (11.5%) as principal fatty acids. The total amount of saturated (SFA), monounsaturated (MUFA) and poly unsaturated fatty acid methyl esters (PUFA) were 41.7%, 25.4%, 32.9% respectively (Figure 5). FAME profile of *D. subspicatus* recorded similar percentages of SFA (39.51 ± 10.92% TFA) and MUFA (21.27 ± 3.07%) in the study of **Maazouzi *et al.* (2012)**. FAME profiles of the current study were also comparable with the findings of **Gressler *et al.* (2014)** in the same microalgal species**. Chaudhary *et al.* (2017**) yielded 47% of SFA and 30% of MUFA and recorded 11% of 18:3 fatty acids when *D. subspicatus* was cultivated in medium with intermediate nitrogen supply and these results justifies the findings of the present study.

A high degree of unsaturation in biodiesel negatively affects the oxidation rate and results in having more iodine value than conventional diesel, which causes storage problems of oil and results in formation of some insoluble compounds affecting the engine performance. Hence long chain saturated fatty acids and mono unsaturated fatty acids are more suitable for biodiesel production to improve oxidative stability (**Jena *et al*., 2012; Mandotra *et al*., 2014**). European standards EN 14214 specifies that ideal biodiesel should contain lesser than 12% of linolenic acid content and 1% of polyunsaturated fatty acids with four or more than four double bonds (≥ 4). Biodiesel yielded from *D. subspicatus* contained 67.1% of saturated and mono unsaturated FAMEs, had 11.3% of linolenic acid and polyunsaturated fatty acids with ≥ 4 double bonds were only 0.4%. These properties make the strain an ideal candidate for quality bio diesel production.

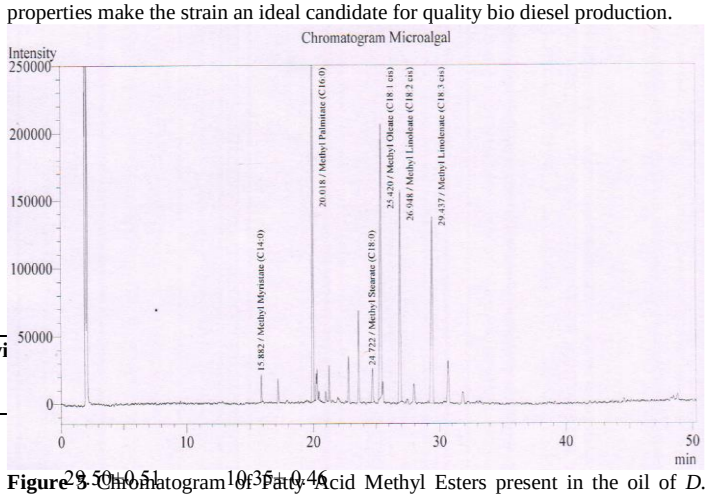


Figure 5 Chromatogram of Fatty Acid Methyl Esters present in the oil of *D. Subspicatus* MB.23.

CONCLUSION

The present investigation was carried out to formulate the cost-effective culture medium for the production of quality biodiesel using the green microalga *D. subspicatus* MB.23. The fertilizer medium having 19:19:19 NPK have the best medium for the growth and biomass. Approximately a two-fold increase of cell density and biomass was obtained in this fertilizer medium over BBM. Based on the criteria like biomass production, lipid yield, fatty acid composition and significant reduction in the nutrient cost (3 USD for 1kg of NPK 19:19:19), it is concluded that culturing of D. subspicatus in 19:19:19 fertilizer medium is cost effective for the large-scale production of quality biodiesel. It is recommended that the medium can also be used for the cultivation of green algae for feed production in commercial aquaculture as there is enhanced production of protein, pigments and ω-6 fatty acids.

Acknowledgments: The authors acknowledge the Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, India for providing the facilities. Authors are also thankful to Prasan solutions (India) private limited, Cochin for fatty acid analysis.

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