Cultivation of freshwater microalga *Scenedesmus* sp. using a low-cost inorganic fertilizer for enhanced biomass and lipid yield

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The potential of an inorganic fertilizer as an alternative nutrient source for the cultivation of *Scenedesmus* sp. IMMTCC-6 was investigated. With a preliminary study at a shake-flask scale, the microalgal cultivation was scaled up in a photobioreactor containing an inorganic fertilizer medium. Microalgae cultured in a shake flask containing 0.1 g L⁻¹ of urea and 1.0 g L⁻¹ of NPK (Nitrogen: Phosphorus: Potassium) fertilizers showed a promising result in biomass productivity. During the scale-up study in a photobioreactor the specific growth rate (µ d⁻¹), biomass yield (g L⁻¹), and total biomass productivity (mg L⁻¹ d⁻¹), was found to be 0.265, 1.19 and 66.1, respectively. The lipid yield (%) as per dry cell weight (DCW) and lipid productivity (mg L⁻¹ d⁻¹) was found to be a maximum of 28.55 and 18.87, respectively, in a stationary phase of the microalgal growth. The fatty acids methyl ester profile was proven to be desirable for biodiesel production.

**Key Words:** biomass; fatty acids; fertilizer; photobioreactor; *Scenedesmus* sp.; total lipid

**Introduction**

Biodiesel has been deemed to be the cleanest fuel alternative to fossil fuel and has received global attention due to its potential as a sustainable and eco-friendly alternative. Microalgae have been demonstrated to be one of the most promising alternative feedstocks for biodiesel production, which may significantly replace petroleum-based fuels (Demirbas and Demirbas, 2011; Wijffels and Barbosa, 2010). It provides several advantages such as better photosynthesis efficiency, higher biomass productivity and lipid content, as compared with conventional energy crops (Li et al., 2008; Liu et al., 2008; Wijffels and Barbosa, 2010). Some algal species can accumulate 50–60% of their dry cell weight as storage lipids which can be increased further with environmental and nutritional stress conditions (Breuer et al., 2012; Hu et al., 2008; Lin et al., 2012). Oil productivity of microalgal culture exceeds the yield of the best oilseed crops, e.g., 12,000 L ha⁻¹ of biodiesel for microalgae cultivated in an open pond production system compared with 1190 L ha⁻¹ for rapeseed (Schenk et al., 2008). It is non-toxic, biodegradable and has a lower emission of greenhouse gases when combusted in a diesel engine (Demirbas, 2009). Further, microalgae can be cultivated throughout the year and also on non-arable land and with non-potable water (Siaut et al., 2011). The potential application of the green microalga, *Scenedesmus* sp., for efficient lipid synthesis for biodiesel production has been reported by various workers (Mandal and Mallick, 2011; Tang et al., 2011; Yoo et al., 2010).

The nutrient composition of the culture medium significantly affects biomass production and total lipid productivity in microalgae. However, the economic feasibility for biodiesel production from microalgae greatly depends on a low cost for its cultivation, and the nutrient source is one of the major limitations in large-scale microalgae cultivation. Several studies have been undertaken to optimize a suitable culture medium for the large-scale cultivation of microalgae with a reasonable cost to achieve a commercially viable algal biodiesel production (Li et al., 2008). The large-scale cultivation of microalgae for biodiesel production requires a substantial amount of low-cost inorganic fertilizers, typically nitrogen, phosphorus and potassium, for an economically viable process. Using an inorganic fertilizer as the nutrient source offers an alternative and convenient way to minimize the cost.

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None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.
Therefore, the objective of the present study was to optimize the concentration of inorganic fertilizer for high biomass productivity of Scenedesmus sp. IMMTCC-6, a high-lipid-content microalga, hence improving the economics of microalgae-derived biodiesel, which has not yet been investigated. Up-scaling experiments were performed using a 12 L self-designed rectangular photobioreactor. Tap water was used, rather than using distilled or sterilized water, in all experimental work. This approach was implemented to promote an easier up-scaling and to reduce the cost of microalgae cultivation at a commercial scale.

Materials and Methods

Microalga strain and inoculum preparation. A wild-type microalgal strain Scenedesmus sp. IMMTCC-6, collected from a local fresh water habitat of Odisha, India (Nayak et al., 2013), was used for this study. The strain was cultivated in a low-cost inorganic fertilizer medium composed of urea and N:P:K (10/26/26) fertilizers which were obtained from local authorized distributors of IFFCO (Indian Farmers Fertilizers Cooperative Limited, India) fertilizers. The urea fertilizer also popularly known as forty-six zero zero (46:0:0) is a highly-concentrated, solid nitrogenous fertilizer containing 46.0% nitrogen. It is completely soluble in water, hence the nitrogen is easily available to plants. The NPK (10:26:26) is used as a complex fertilizer with three major nutrients, viz. nitrogen, phosphorus and potassium in the proportion of 10% (nitrogen), 26% (P2O5) and 26% (K2O). The strain was cultivated at a pH of 6.8.

Initially, microalgae were grown in a modified Bold Basal medium (Stein, 1973) in distilled water under aseptic conditions. The microalgae cells were harvested by centrifugation at 3,000 rpm for 15 min (REMI Cooling Centrifuge, C-24 BL, India) and resuspended then inoculated to the culture medium to initiate the microalgae cultivation. The optimum nutrient concentration (NPK; 1.0 g L–1 with urea; 0.1 g L –1) obtained in a shake-flask study was used as the nutrient concentration in the photobioreactor study. Up-scaling experiments were performed from the bioreactor at regular intervals to determine the microalgae biomass yield, pH, and lipid content, of the microalgal biomass.

Photobioreactor (PBR) operation. A self-designed simple rectangular photobioreactor with a working volume of 12 L was used for microalgal cultivation (Nayak et al., 2011) and maintained at a temperature of 25 ± 1°C. A fluorescent lamp in an enclosed glass tube submerged in the culture medium was used to provide continuous light (24 h) of around 60 μmol photons m–2 day–1 at the surface of the photobioreactor. Aeration was effected continuously by sterile air provided via bubbling from the bottom of the photobioreactor with a rate of 6 L min–1, i.e., 0.5vvm (volume gas per volume of broth per minute). The cultures were continuously mixed with the help of an agitator. Water was added to the culture medium daily to compensate for evaporation loss during microalgae cultivation. One batch culture was run for a period of 18 days.

Dry weight measurement. The biomass growth of microalgae cultures was monitored by measuring the absorbance at 680 nm (OD680) using a Cecil UV-vis spectrophotometer. Microalgal cells were collected by centrifugation (3000 rpm for 10 min) at 4°C and the algal cell pellets were washed twice with distilled water, and subjected to oven drying at 65°C for 24 h for an estimation of dry biomass (Takagi and Karseno, 2006). Further, a regression equation of cell density (OD680) versus dry weight (Nayak et al., 2013) was used for the determination of the biomass concentration (dry weight per liter). The correlations established in the Regression Equation of the Cell Density was Biomass = (OD – 0.0888)/3.9094 (R2 = 0.994).

Kinetic and yield parameters. The specific growth rate (μ, d–1) of microalgal was calculated using the equation:

$$\mu = \ln(W_t/W_0)/\Delta t,$$  

where $W_t$ and $W_0$ are the biomass concentrations (g L–1) at the end and at the beginning of a batch run, respectively, and $\Delta t$ is the cultivation time in days (Chiu et al., 2009).

The biomass productivity, $P_B$ (mg L–1 d–1) was calculated using the following equation:

$$P_B = C_B \times 1000/t,$$  

where $C_B$ is the biomass concentration (g L–1) and $t$ is the cultivation time (days).

**Table 1.** Composition of inorganic fertilizer in different culture flasks.

<table>
<thead>
<tr>
<th>Flask No.</th>
<th>Urea (g/L)</th>
<th>NPK (g/L)</th>
<th>Total Nitrogen, N (g/L)</th>
<th>Total Phosphorus, P (g/L)</th>
<th>Total Potassium, K (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1</td>
<td>0</td>
<td>0.046</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SF-1</td>
<td>0.1</td>
<td>0.25</td>
<td>0.071</td>
<td>0.065</td>
<td>0.065</td>
</tr>
<tr>
<td>SF-2</td>
<td>0.1</td>
<td>0.5</td>
<td>0.096</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>SF-3</td>
<td>0.1</td>
<td>0.75</td>
<td>0.121</td>
<td>0.195</td>
<td>0.195</td>
</tr>
<tr>
<td>SF-4</td>
<td>0.1</td>
<td>1</td>
<td>0.146</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>SF-5</td>
<td>0.1</td>
<td>1.25</td>
<td>0.171</td>
<td>0.325</td>
<td>0.325</td>
</tr>
</tbody>
</table>
**Results and Discussion**

**Growth and biomass productivity of microalgae cultured in the shake flask**

It is observed that the use of NPK and urea as a sole source of nutrients (nitrogen, phosphorus and potassium) proved to be beneficial with respect to the growth and extraction of the total lipid from the microalgal biomass was carried out using the modified method of Bligh and Dyer (1959). The algal lipids were extracted in a mixture of chloroform and methanol (2:1, v/v), and separated into chloroform and an aqueous methanol layer by the addition of methanol and water to give a final solvent ratio of 2:2:1 of chloroform: methanol: water. The chloroform layer containing the lipid was washed with a 1% NaCl solution. It was collected and evaporated to dry using a rotary evaporator. The lipid content was determined gravimetrically.

The fatty acid composition of microalgal fatty acid methyl esters were determined by an Acid Hydrolysis method (Hungerford, 1995). Microalgal oil was taken in a 50 ml RB flask and 15 ml of 2% sulfuric acid in methanol solution was added to this, and the contents were refluxed for 3 h at 70°C. On completion of the time period, the contents were diluted with water and the organic phase was extracted with ethyl acetate. The ethyl acetate phase was thoroughly washed with water and dried over anhydrous sodium sulfate. Ethyl acetate was evacuated on a rotary evaporator to recover the fatty acid methyl ester. An Agilent 6890 N series gas chromatograph equipped with a flame ionization detector (FID) was used for fatty acid analysis. A fused silica capillary column (DB-225, 0.25 μm, 30 m × 0.32 mm i.d.) was used for the analysis. The oven temperature was programmed from 170°C to 225°C at 1°C min⁻¹. The injector and detector temperature were kept at 250°C and 270°C, respectively, with a split ratio of 50:1. Nitrogen was used as a carrier gas with a flow rate of 1 mL min⁻¹. The area percentages were recorded with the Agilent chemstation data processing system. Statistical analysis. The results were expressed as the mean ± standard deviation (SD) of three replicates. The Student’s t-test was used to evaluate differences between groups of discrete variables. A value of p < 0.05 was considered statistically significant.

**Table 2.** The specific growth, biomass yield, total biomass productivity, and CO₂ consumption rate, of *Scenedesmus* sp. IMMTCC-6 at different concentrations of fertilizer.

<table>
<thead>
<tr>
<th>Shake flask No.</th>
<th>Specific growth rate (µ, d⁻¹)</th>
<th>Biomass yield (Cₚ, g L⁻¹)</th>
<th>Total biomass productivity (Pᵥ, mg L⁻¹ d⁻¹)</th>
<th>CO₂ consumption rate (P_CO₂, mg L⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.19</td>
<td>0.32 ± 0.01</td>
<td>17.8 ± 0.8</td>
<td>33.5 ± 1.5</td>
</tr>
<tr>
<td>SF-1</td>
<td>0.22</td>
<td>0.55 ± 0.02</td>
<td>30.4 ± 1.1</td>
<td>57.2 ± 2.1</td>
</tr>
<tr>
<td>SF-2</td>
<td>0.236</td>
<td>0.7 ± 0.02</td>
<td>38.9 ± 1.02</td>
<td>73.1 ± 1.9</td>
</tr>
<tr>
<td>SF-3</td>
<td>0.243</td>
<td>0.8 ± 0.01</td>
<td>44.4 ± 0.82</td>
<td>83.5 ± 1.5</td>
</tr>
<tr>
<td>SF-4</td>
<td>0.25</td>
<td>0.9 ± 0.02</td>
<td>50 ± 0.91</td>
<td>94 ± 1.7</td>
</tr>
<tr>
<td>SF-5</td>
<td>0.246</td>
<td>0.84 ± 0.02</td>
<td>46.6 ± 0.87</td>
<td>87.7 ± 1.6</td>
</tr>
<tr>
<td>PBR*</td>
<td>0.265</td>
<td>1.19 ± 0.03</td>
<td>66.1 ± 1.98</td>
<td>124.3 ± 3.7</td>
</tr>
</tbody>
</table>

Data are represented as mean ± standard.

*Photobioreactor having NPK, 1 g L⁻¹ with urea 0.1 g L⁻¹ in a culture medium.
biomass productivity of *Scenedesmus* sp. IMMTCC-6. Optimum nutrient supplement in a culture medium for microalgae cultivation plays a major role in high biomass productivity (Xin et al., 2010). The applications of fertilizers are deemed to be a cost effective substitute for the microalgal nutrients requirement. Figure 1 illustrates the growth rate of *Scenedesmus* sp. IMMTCC-6 in six different fertilizer concentrations in each flask. Many researchers reported that the growth rate was accelerated, compared to a control having only urea. It is obvious from the observed data that the growth rate of *Scenedesmus* sp. IMMTCC-6 increased from 0.19 d\(^{-1}\) to 0.25 d\(^{-1}\) when the fertilizer (NPK) was shifted from control (0 g L\(^{-1}\)) to fertilizer with 1 g L\(^{-1}\). In tandem with this, the biomass productivity of *Scenedesmus* sp. IMMTCC-6 was significantly increased from 17.8 mg L\(^{-1}\) d\(^{-1}\) to 50 mg L\(^{-1}\) d\(^{-1}\). In addition, *Scenedesmus* sp. IMMTCC-6 cultivated in the fertilizer (NPK, 1 g L\(^{-1}\)) had a shorter lag phase and attained an early stationary phase on the 18th day, whereas *Scenedesmus* sp. IMMTCC-6 cultivated in an NPK free condition exhibited a prolonged lag phase. There was approximately the same biomass growth pattern in flask SF-5 (having NPK, 1.25 g L\(^{-1}\)) compared to that of flask SF-4 (having an NPK, 1 g L\(^{-1}\)). The culture having fertilizer (NPK, 1 g L\(^{-1}\)) has generated a higher biomass productivity and thus the addition of extra fertilizer will lead to an elevated cost for microalgal cultivation. Hence, the microalgae supplemented with NPK fertilizer 1 g L\(^{-1}\) showed a higher biomass yield in a cost effective manner. On the other hand, the microalga, *Scenedesmus* sp. IMMTCC-6, yielded less biomass with the addition of an excess amount of fertilizer (NPK, 1.25 g L\(^{-1}\)) and also showed a less specific growth rate.

Initially, the fertilizer requirements for algal growth were optimized in a shake flask by keeping the urea concentration 0.1 g L\(^{-1}\) with a variation in the NPK concentration in each flask. Many researchers reported that urea (0.1 g L\(^{-1}\)) in the form of a nitrogen supply, appears
to be the most significant for microalgae growth (Arunugam et al., 2013; Goswami and Kalita, 2011; Makareviceni et al., 2011). We are of the opinion that the combination of a NPK fertilizer with urea, in a potable tap water medium, effectively supplied the nutritional requirement for microalgae *Scenedesmus* sp. IMMTCC-6 growth and improved the biomass and lipid productivity. Thus, complex media components are avoided thereby making the cultivation process more economical.

As summarized in Table 2, a maximum specific growth rate (0.25 d⁻¹), biomass yield (0.9 g L⁻¹), total biomass productivity (50 mg L⁻¹ d⁻¹) and the maximum CO₂ consumption rate (P_{CO₂}) of 7.3 to 7.9 during cultivation time. The culture flask SF-1 (7.5 to 9.04) and the culture flask SF-2 (7.4 to 8.3) show a high pH range during cultivation time. The initial pH during the 1st day of cultivation time also shows a significant difference in the control flask (without NPK) having a pH 8.4 as compared with the other flask (with NPK) (7.3 to 7.5). The addition of extra fertilizer, NPK leads to a decrease in the pH of the culture solution (Fig. 2). This may be as a result of the fact that urea is basic in solution and NPK fertilizer might be slightly acidic. This finding is in close agreement with the results reported by Amenaghawon et al. (2013).

### pH change of microalgae

Microalgae cultivated with a control condition having no fertilizer (NPK) maintained a high pH up to the 14th day of cultivation time, and then its pH decreased with an increase of cultivation time. Culture flasks; SF-3, SF-4 and SF-5, show a similar pH change pattern during the cultivation time and a pH maintained within the range of 7.3 to 7.9 during cultivation time. The culture flask SF-1 (7.5 to 9.04) and the culture flask SF-2 (7.4 to 8.3) show a high pH range during cultivation time. The initial pH during the 1st day of cultivation time also shows a significant difference in the control flask (without NPK) having a pH 8.4 as compared with the other flask (with NPK) (7.3 to 7.5). The addition of extra fertilizer, NPK leads to a decrease in the pH of the culture solution (Fig. 2). This may be as a result of the fact that urea is basic in solution and NPK fertilizer might be slightly acidic. This finding is in close agreement with the results reported by Amenaghawon et al. (2013).

### Growth and pH change of microalgae cultured in the photobioreactor

The optimized nutrient concentration (NPK; 1.0 g L⁻¹ with Urea; 0.1 g L⁻¹) obtained from a shake-flask study was applied for cultivating microalgae in the photobioreactor study. A pre-cultured *Scenedesmus* sp. IMMTCC-6 was inoculated in the photobioreactor to reach an initial biomass concentration of 0.01 g L⁻¹ as a batch culture. The growth in the photobioreactor reached an early stationary phase on the 15th day of cultivation time (Fig. 3). Better growth was observed in the photobioreactor due to proper aeration and agitation of the system. As indicated in Table 2, a maximum specific growth rate (0.265 d⁻¹), biomass yield (1.19 g L⁻¹), total biomass productivity (66.1 mg L⁻¹ d⁻¹), and the maximum CO₂ consumption rate (P_{CO₂}) of 124.3 mg L⁻¹ d⁻¹, were observed in the photobioreactor. A change in pH was observed during the growth of microalgae *Scenedesmus* sp. IMMTCC-6 in the photobioreactor (Fig. 3). The increase in the culture pH is mainly due to an increase in algal biomass. Increased algal biomass rapidly absorbs dissolved CO₂ from the (carbonate ion of the) culture medium for its photosynthesis. This process leads to an increase in hydroxide ion and a decrease in carbonate ion in the solution, which causes a rise in the culture pH. Similar findings have also been reported in previous studies (Kumar et al., 2015; Nayak et al., 2013). Despite an increase in the culture pH, *Scenedesmus* sp. IMMTCC-6 was found to grow well without inhibition. This shows that the culture pH had no significant effects on the biomass yield. This observation is consistent with those in the case of other green algal species also (Dayananda et al., 2007; Moheimani, 2013; Nayak et al., 2013). In the present study, the increase of the culture pH was controlled to some extent with the continuous aeration and agitation.

### Biomass and lipid productivity

The microalgal cells were collected from logarithmic, early stationary phase and stationary phase of growth to measure biomass and lipid productivity. The biomass yield (g/L) and biomass productivity (mg L⁻¹ day⁻¹) of the photobioreactor was 1.19 and 66.1, respectively, on the 18th day of cultivation (Fig. 4). In this context, the microalga *Scenedesmus* sp. IMMTCC-6 was found to be better in comparison with previously reported results of related strains (Hodaifa et al., 2009; Matsunaga et al., 2009; Morais and Costa, 2007).

The result shown in Fig. 5 indicates that the lipid accumulation in microalgal cells was associated with growth phases. Lipid accumulation increases as the culture approaches the stationary phase. This lipid accumulation may be due to nutrient depletion in the culture medium. Roessler (1988) reported that the nutrient deficiency induced an increase in the rate of lipid synthesis in a diatom, *Cyclotella cryptica*, and resulted in lipid accumulation in the cells. Similar results have been reported in the green algae *Chlorella* and *Chlamydomonas* (e.g., Shen et al., 2015). Finally, microalgae total lipid was extracted and lipid yield in *Scenedesmus* sp. IMMTCC-6 was 28.55%, with a lipid productivity of 18.87 mg L⁻¹ day⁻¹ on the 18th day of cultivation time.

### Table 3. Fatty acid composition (% of total fatty acids) of *Scenedesmus* sp. IMMTCC-6 in a photobioreactor.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>6th day</th>
<th>12th day</th>
<th>18th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated Fatty Acids (SFA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0 (Myristic acid)</td>
<td>1.1</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>C15:0 (Pentadecylic acid)</td>
<td>0.6</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>C16:0 (Palmitic acid)</td>
<td>38.3</td>
<td>35.4</td>
<td>34.7</td>
</tr>
<tr>
<td>C17:0 (Margaric acid)</td>
<td>1.2</td>
<td>1.8</td>
<td>2.1</td>
</tr>
<tr>
<td>C18:0 (Stearic acid)</td>
<td>7.1</td>
<td>7.7</td>
<td>7.1</td>
</tr>
<tr>
<td>C20:0 (Arachidic acid)</td>
<td>0.1</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>C22:0 (Behenic acid)</td>
<td>1.1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Total SFAs</td>
<td>49.5</td>
<td>47.3</td>
<td>46.2</td>
</tr>
<tr>
<td>Monounsaturated Fatty acids (MUFA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1 (Palmitoleic acid)</td>
<td>4.2</td>
<td>5.3</td>
<td>3.9</td>
</tr>
<tr>
<td>C18:1 (Oleic acid)</td>
<td>12.9</td>
<td>12.6</td>
<td>13.3</td>
</tr>
<tr>
<td>C20:1 (Eicosenoic acid)</td>
<td>0.8</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>C22:1 (Docosenoic acid)</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>18.2</td>
<td>18.7</td>
<td>18.2</td>
</tr>
<tr>
<td>Polyunsaturated Fatty acids (PUFA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:2 (Hexadecadienic acid)</td>
<td>3.9</td>
<td>2.6</td>
<td>4.1</td>
</tr>
<tr>
<td>C16:3 (Hexadecatrienic acid)</td>
<td>2.7</td>
<td>5.1</td>
<td>3.9</td>
</tr>
<tr>
<td>C18:2 (Linoleic acid)</td>
<td>10.4</td>
<td>11.9</td>
<td>10.1</td>
</tr>
<tr>
<td>C18:3 (Linolenic acid)</td>
<td>9.5</td>
<td>8.7</td>
<td>14.2</td>
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<tr>
<td>C20:2 (Eicosadienic acid)</td>
<td>0.9</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Total PUFAs</td>
<td>27.4</td>
<td>28.7</td>
<td>32.5</td>
</tr>
<tr>
<td>Hydrocarbons and Un-identified</td>
<td>4.9</td>
<td>5.3</td>
<td>3.1</td>
</tr>
</tbody>
</table>
**Fatty acid composition of total lipids**

The fatty acid compositions of *Scenedesmus* sp. IMMTCC-6 under different culture conditions are shown in Table 3. Fatty acid composition differed in three different culture stages. Microalgae cultured on the 18th day, i.e., in the stationary phase, revealed high percentage of PUFA compared with the 12th day and 6th day of cultivation time (Table 3); the former with a higher PUFA yield being more desirable feedstock for biodiesel production. The microalga contained a high percentage of saturated fatty acids in all the three cultivation times of growth i.e., from 46.2% to 49.5%. The high saturated fatty acid methyl ester (FAME) indicated them to be a source of biodiesel with higher oxidation stability (Nascimento et al., 2013). As the cultivation time increased, the total PUFA content increased from 27.4% (on the 6th day) to 32.5% (on the 18th day) during the cultivation period, whereas the SFA content decreased from 49.5 (on the 6th day) to 46.2% (on the 18th day). During the experiment, less variation in monounsaturated fatty content was observed. The microalga contained a high amount of saturated fatty acid (46.2%) followed by PUFA (32.5%) and MUFA (18.2%) on the 18th day of the cultivation period, where lipid productivity was high. In general, saturated fatty acids have higher melting points and unsaturated fatty acids have lower melting points, which recommend them for utilization in cold regions.

From the above observations, it is inferred that *Scenedesmus* sp. IMMTCC-6 grows fast, yields a good biomass and contains about 28.5% lipid with a high percentage of saturated fatty acids under optimal conditions. These properties make it a suitable candidate for biodiesel production. The results of this study show that it is feasible to use an inorganic fertilizer as an alternative nutrient source to cultivate *Scenedesmus* sp. IMMTCC-6 for biodiesel production. The application of NPK (1 g L⁻¹) along with urea (0.1 g L⁻¹) could be cost-effective for the large-scale cultivation of *Scenedesmus* sp. IMMTCC-6 in a raceway pond with a higher biomass yield.

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**References**


Nascimento, I. A., Marques, S. S. I., Cabanelas, I. T. D., Pereira, S. A., Souza, C. O. et al. (2013) Screening microalgae strains for biomass production and CO₂ mitigation and purification of *Scenedesmus* sp. strain IMMTCC-6 under different culture conditions are shown in Table 3. Fatty acid composition differed in three different culture stages. Microalgae cultured on the 18th day, i.e., in the stationary phase, revealed high percentage of PUFA compared with the 12th day and 6th day of cultivation time (Table 3); the former with a higher PUFA yield being more desirable feedstock for biodiesel production. The microalga contained a high percentage of saturated fatty acids in all the three cultivation times of growth i.e., from 46.2% to 49.5%. The high saturated fatty acid methyl ester (FAME) indicated them to be a source of biodiesel with higher oxidation stability (Nascimento et al., 2013). As the cultivation time increased, the total PUFA content increased from 27.4% (on the 6th day) to 32.5% (on the 18th day) during the cultivation period, whereas the SFA content decreased from 49.5 (on the 6th day) to 46.2% (on the 18th day). During the experiment, less variation in monounsaturated fatty content was observed. The microalga contained a high amount of saturated fatty acid (46.2%) followed by PUFA (32.5%) and MUFA (18.2%) on the 18th day of the cultivation period, where lipid productivity was high. In general, saturated fatty acids have higher melting points and unsaturated fatty acids have lower melting points, which recommend them for utilization in cold regions.

From the above observations, it is inferred that *Scenedesmus* sp. IMMTCC-6 grows fast, yields a good biomass and contains about 28.5% lipid with a high percentage of saturated fatty acids under optimal conditions. These properties make it a suitable candidate for biodiesel production. The results of this study show that it is feasible to use an inorganic fertilizer as an alternative nutrient source to cultivate *Scenedesmus* sp. IMMTCC-6 for biodiesel production. The application of NPK (1 g L⁻¹) along with urea (0.1 g L⁻¹) could be cost-effective for the large-scale cultivation of *Scenedesmus* sp. IMMTCC-6 in a raceway pond with a higher biomass yield.