

1 **Growth kinetic and fuel quality parameters as selective criterion for screening biodiesel**
2 **producing cyanobacterial strains**

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24 **Abstract**

25 The efficiency of cyanobacterial strains as biodiesel feedstock varies with the dwelling habitat.
26 Fourteen indigenous heterocystous cyanobacterial strains from rice field ecosystem were
27 screened based on growth kinetic and fuel parameters. The highest biomass productivity was
28 obtained in *Nostoc punctiforme* MBDU 621 (19.22 mg L⁻¹d⁻¹) followed by *Calothrix* sp. MBDU
29 701 (13.43 mg L⁻¹d⁻¹). While Lipid productivity and lipid content was high in *Nostoc*
30 *spongiaeforme* MBDU 704 (4.45 mg L⁻¹d⁻¹ and 22.5 % dwt) followed by *Calothrix* sp. MBDU
31 701 (1.54 mg L⁻¹d⁻¹ and 10.75 % dwt). Among the tested strains, *Nostoc spongiaeforme* MBDU
32 704 and *Nostoc punctiforme* MBDU 621 were selected as promising strains for good quality
33 biodiesel production by Preference Ranking Organization Method for Enrichment Evaluation
34 (PROMETHEE) and Graphical Analysis for Interactive Assistance (GAIA) analysis.

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37 **Keywords:** Cyanobacteria, Biodiesel, FAME, Fuel quality parameters, Growth kinetics,

38 **PROMETHEE-GAIA**

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47 **1. Introduction**

48 Fast depletion of fossil fuels with exploding population mandates global energy agendas towards
49 the development of renewable sources of fuel. Among different generations of renewable
50 feedstock, photosynthetic microalgae serve as a promising biomass for a diverse number of
51 products such as fine chemicals, nutraceuticals, aquaculture, feed and cosmetics in addition to
52 biofuels (Gerardo et al., 2015). Despite much research drive for the past two decades, microalgal
53 biofuel has not yet been a commercial reality. For the past two years, collapse in the oil prices
54 imposes large economic pressure on biofuel production (Cate and Ball, 2016). In order to make
55 microalgal biofuels commercially feasible and practically viable, microalgal biomass must be
56 processed similar to petroleum refinery for extracting multiple products in addition to biofuel, in
57 a biorefinery concept (Maurya et al., 2016). Many up- and downstream processes have
58 successfully been integrated during the conversion of microalgal biomass. For example,
59 integrating the upstream microalgal cultivation with wastewater treatment reduces overall
60 residual waste component and favors sustainable economy (Mohan et al., 2016). During
61 downstream processing, high volumes of products such as proteins and carbohydrates, and low
62 volume high value products such as astaxanthin, β -carotene, and polyunsaturated fatty acids such
63 as eicosapentaenoic acid and docosahexaenoic acid have also been co-extracted from microalgal
64 biomass, and have significant market demand (Gerardo et al., 2015). Therefore, a biorefinery
65 should be able to produce a gamut of marketable products and energy in a sustainable fashion
66 (Gravitis, 2008). Utilisation of additional products help to subsidize the overall fuel costs (Chuck
67 et al., 2015).

68 Owing to its simple growth requirements, increased growth rate and ease of genetic
69 engineering with developed molecular tools, cyanobacteria serve as an attractive candidate over

70 eukaryotic microalgae in terms of biomass feedstock utilization. Until recently, many researchers
71 have successfully co-produced various valuable products in metabolically engineered
72 cyanobacteria (Angermayr et al., 2015). However, only little attempt is made in the exploration
73 of natural cyanobacterial species with the potency of producing different commercially important
74 products. Knowledge of these properties is an important criteria in the selection of most suitable
75 strains which can be exploited successfully at commercial level. With this in view, the present
76 study is carried out to explore the lipid productivity, lipid content and biodiesel properties of
77 phytohormone producing cyanobacterial strains.

78 **2. Methods**

79 *2.1. Cultivation of cyanobacterial strains*

80 The fourteen cyanobacterial strains, *Scytonema bohneri* MBDU104, *Calothrix* sp. MBDU901,
81 *Nostoc spongiaeforme* MBDU704, *Nostoc commune* MBDU101, *Nostoc muscorum* MBDU702,
82 *Nostoc* sp MBDU804, *Anabaena spiroides* MBDU903, *Nostoc Punctiforme* MBDU621,
83 *Calothrix* sp MBDU701, *Aphanothece stagnina* MBDU803, *Anabaena variabilis* MBDU103,
84 *Nostoc* sp MBDU001, *Nostoc commune* MBDU703, and *Nostoc microscopicum* MBDU102,
85 characterised previously for phytohormone production (Gayathri et al., 2017) were grown in BG-
86 11_o medium in 250 mL Erlenmeyer flask at $28 \pm 1^\circ\text{C}$ under continuous light ($50 \mu\text{E m}^{-2} \text{s}^{-1}$)
87 (Rippka et al., 1979).

88 *2.2. Growth kinetic parameters*

89 The growth kinetic parameters of fourteen strains were determined after harvesting the cells in
90 their stationary growth phase. All the measurements were performed in triplicates. The
91 parameters analyzed included:

- 92 - **Biomass productivity (Pb)** indicates the amount of dry biomass produced ($\text{g L}^{-1} \text{ day}^{-1}$).
93 For Pb determination, algal suspensions were centrifuged at 3000 g for 10min at room
94 temperature and the resulting pellets were washed with deionized water, lyophilized at
95 $-40\text{ }^{\circ}\text{C}$ for 48 h and their dry weights were determined gravimetrically.
- 96 - **Total lipid content (Lc)**, reported as percentage of the total biomass (% dwt), and
97 determined based on the method by Folch et al., (1957).
- 98 - **Volumetric lipid productivity (Lp, $\text{mg L}^{-1} \text{ day}^{-1}$)**, was calculated according to the
99 following equation (Liu et al., 2011b).

$$100 \quad L_p = P_b \times L_c \quad (1)$$

101

102 *2.3. Total lipid extraction*

103 The total lipid from the tested strains was extracted according to Folch et al., (1957). 40 mg of
104 freeze-dried biomass was extracted with 10 mL of chloroform:methanol (2:1) using pestle and
105 mortar. The extract was filtered through Whatman No. 1 filter paper. To the filtrate three
106 volumes of distilled water was added. The filtrate was then vortexed for 5 mins, and allowed to
107 undergo phase separation for 15 mins. Lower phase containing essentially all extracted lipids
108 were transferred into a weighed, clean glass vials and allowed to dry in a rotary evaporator to
109 remove solvent mixture. The dried lipid was quantified and expressed as percent on dry weight
110 basis.

111 *2.4. FAME analysis by GC*

112 Fatty acid profile was analysed by preparation of fatty acids methyl ester (FAME) and Gas
113 Chromatography–Mass Spectrometry analysis. FAME was prepared directly using the
114 transesterification method described by Indarti et al. (2005), with minor modification. Dried

115 algae samples (about 30 mg) were weighed onto clean glass vials and allowed to react directly
116 with 10 mL mixture of methanol, concentrated sulfuric acid and chloroform (4.25:0.75:5).
117 Transesterification was carried out in a 90°C water bath for 90 min. On completion of the
118 reaction, the vials were cooled down to room temperature and then, 1 mL of distilled water was
119 added into the mixture and thoroughly vortexed for 5 min. After the formation of two phases, the
120 lower phase containing FAME was transferred to a clean glass vial and dried. The samples were
121 analyzed via GC (Shimadzu, QP 2010, Japan) with FID detector. The oven temperature was set
122 at 80 °C, and held for 5 min, then raised to 290 °C at a rate of 4 °C/min, and held at 290 °C for 5
123 min, while the injector and detector temperature were set at 270 °C and 280 °C, respectively. The
124 SP-2560 column (Supelco, USA) (100 m × 0.25 mm I.D. × 0.20 µm film thickness) was used for
125 the analysis of FAME. The carrier gas (helium) was controlled at 2 mL/ min. Concentrations of
126 individual FAMEs were determined by comparing sample peak areas with C-8 to C-24 FAME
127 mixture from Supelco Analytical (Bellefonte, PA).

128 2.5. Calculation of fuel properties

129 To screen the suitable indigenous cyanobacterial strains for biodiesel production based on FAME
130 profile, the following 15 biodiesel properties were calculated: i) iodine value (IV)- Equation (2)
131 ii) saponification value (SV) – Equation (3) iii) cloud point (CP)- Equation (4) iv) pour point
132 (PP)- Equation (5) v) cetane number (CN)- Equation (6) vi) Degree of unsaturation (DU)-
133 Equation (7) vii) long chain saturation factor (LCSF)- Equation (8) viii) cold filter plugging point
134 (CFPP)- Equation (9) ix) allylic position equivalent (APE)- Equation (10) x) bisallylic position
135 equivalent (BAPE)- Equation (11) xi) kinematic viscosity (ν)- Equation (12) xii) density (ρ)-
136 Equation (13) xiii) high heating value (HHV)- Equation (14) (Anahas and Muralitharan, 2015).

137 xiv) Oxidative stability (OS) - Equation (15) (Wang et al., 2012) xv) flash point temperature (FP)
138 - Equation (16) (Agarwal et al., 2010).

139

$$140 \quad IV = \sum (254 \times DN) / M \quad (2)$$

141 *D* is the number of double bonds, *M* is the molecular weight and *N* is the percentage of each fatty
142 acid.

143

$$144 \quad SV = \sum (560 \times N) / M \quad (3)$$

145

$$146 \quad CP = (0.526 \times C16) - 4.992 \quad (4)$$

147

$$148 \quad PP = (0.571 \times C16) - 12.240 \quad (5)$$

149

$$150 \quad CN = 46.30 + (5458 / SV) - (0.225 \times IV) \quad (6)$$

151

$$152 \quad DU = MUFA + (2 \times PUFA) \quad (7)$$

153 MUFA – monounsaturated fatty acid, PUFA-polyunsaturated fatty acid (in WT %)

154

$$155 \quad LCSF = (0.1 \times C16) + (0.5 \times C18) + 1 \times C20 + (1.5 \times C22) + 2 \times C24 \quad (8)$$

156

$$157 \quad CFPP = (3.1417 \times LCSF) - 16.477 \quad (9)$$

158

$$159 \quad APE = \sum (apn \times Acn) \quad (10)$$

160 $BAPE = \sum (b_{pn} \times A_{cn})$ (11)

161 where a_{pn} and b_{pn} are the numbers of allylic and bisallylic positions in a specific FA,
162 respectively, and A_{cn} is the amount (mass percent) of each FA in the mixture.

163

164 $\ln(v_i) = -12.503 + 2.496 \times \ln(M_i) - 0.178 \times N$ (12)

165 $\rho_i = 0.8463 + 4.9/M_i + 0.0118 \times N$ (13)

166 $HHV_i = 46.19 - 1794/M_i - 0.21 \times N$ (14)

167 where v_i is the kinematic viscosity of at 40 °C in mm^2/s ; ρ_i is the density at 20 °C in g/cm^3 ; and

168 HHV_i is the higher heating value in MJ/kg of i th FAME.

169 $OS = -0.03844 \times DU + 7.770$ (15)

170

171 $FP = 205.226 + 0.083 \times C16:0 - 1.723 \times C18:0$
172 $-0.5717 \times C18:1 - 0.3557 \times C18:2 - 0.467 \times C18:3 - 0.2287 \times C22$ (16)

173 2.6. Biochemical analysis

174 Freeze-dried biomass (5mg) was suspended by vortexing in 0.2ml of 24% (w/v) TCA and
175 incubated 95°C for 15 mins in a screw capped micro-centrifuge tubes and allowed to cool at
176 room temperature. TCA precipitation was carried out by adding 0.6 mL of distilled water and
177 cooling the suspension at room temperature. After centrifugation at 15,000 rpm for 20 mins at
178 4°C, the supernatant was discarded and the precipitate was re-suspended in 0.5mL of Lowry
179 reagent 'D'. This alkaline suspension was incubated at 55°C for 3 hr followed by centrifugation
180 at 15,000 rpm for 15 mins at room temperature. The pellet was discarded and the supernatant
181 was used for protein and carbohydrate estimation (Slocombe et al., 2013).

182 The carbohydrate content, including reducing sugar and total carbohydrates was
183 determined using a dinitro salicylic acid method (Miller, 1959) and Anthrone method (Hedge
184 and Hofreiter, 1962), respectively. Protein estimation was carried out by Lowry method against
185 BSA as a standard (Lowry et al., 1951). Data for all experiments represent the average of three
186 replicates.

187 2.7. Selection of suitable strains for biodiesel production using MCDA-PROMETHEE

188 Among the fourteen cyanobacterial strains, the highest lipid yielding strain was selected based on
189 the Preference Ranking Organization Method for Enrichment Evaluation (PROMETHEE)
190 analysis by choosing the linear preference and appropriate threshold value for both p-preference
191 threshold (smallest difference enough to generate a full preference) and q-indifference threshold
192 (largest difference that is considered negligible by the decision maker and it is enough to
193 generate a full preference) (Brans and Mareschal, 2005) for the criteria. The criteria taken for
194 initial screening was biomass productivity, lipid productivity and lipid content. The selected best
195 five strains were further analyzed for FAME yield and biodiesel properties by gas
196 chromatography. Based on the FAME yield, fifteen biodiesel properties were included as criteria
197 and calculated along with saturated fatty acid (SFA), poly unsaturated fatty acid (PUFA), and
198 mono unsaturated fatty acid (MUFA) to select a best strain. The threshold values were set as per
199 Table. 4. by giving equal weight to all biodiesel quality parameters. This MCDA –
200 PROMETHEE and strain selection was well reported for biodiesel producing microbes (Islam et
201 al., 2013; Anahas and Muralitharan, 2015).

202 3. Results and discussion

203 The tested cyanobacterial strains belonging to five different genera ie. *Nostoc*, *Calothrix*,
204 *Scytonema*, *Anabaena*, and *Aphanothece* were isolated and identified at morphological and

205 molecular level in our previous study (Gayathri et al., 2017). To evaluate whether an algal strain
206 is suitable for biodiesel production, the key criteria such as lipid content, lipid productivity, TAG
207 content in total lipid and suitable fatty acid composition (Talebi et al., 2013) were analysed.
208 Typically, lipid content was reported as percentage dry weight (% DCW). Lipid productivity was
209 influenced by both biomass accumulation and lipid content (Hoekman et al., 2012). For all the
210 tested cyanobacterial strains, biomass productivity varied from 19.22 to 2.9 mg/L/ day, lipid
211 productivity from 4.45 to 0.1 mg/L/ day and lipid content from 22.5 to 1.49 in terms of % dwt
212 (Fig. 1). The highest biomass productivity was shown by *Nostoc punctiforme* MBDU 621 (19.22
213 mg/L/day) followed by *Calothrix* sp. MBDU 701 (13.427 mg/L/day), *Scytonema bohneri* MBDU
214 104 (12.62 mg/L/day), *Nostoc spongiaeforme* MBDU 704 (11.9) and *Nostoc* sp. MBDU001
215 (11.48 mg/L/day). With the exception of *Nostoc punctiforme* MBDU 621, the leading biomass
216 producing cyanobacterial strains showed high lipid productivity and lipid content. For example
217 *Nostoc spongiaeforme* MBDU 704 showed a lipid productivity and lipid content of 4.452
218 mg/L/day and 22.5 % dwt, respectively, followed by *Calothrix* sp. MBDU 701 and *Scytonema*
219 *bohneri* MBDU 104. Similar to our results the lipid productivity of 4.39-7.13 mg/L/day was
220 reported for single cultures of tested cyanobacterial and algal strains and reported an increase in
221 lipid productivity during dual-species cultures (Gonçalves et al., 2016). Compared to the lipid
222 content of previously reported heterocystous cyanobacterial strains (4.68 to 18.65 % dwt)
223 (Anahas and Muralitharan, 2015), the tested cyanobacterial strains in this study showed higher
224 lipid content of 1.495 to 22.5 % dwt. Similarly, our tested strains showed an increased lipid
225 content under normal extraction method (chloroform:methanol; 2:1 v/v) than the
226 cyclohexance:methanol (2:1, v/v) extraction reported as best solvent system for *Microcystis*
227 *aeruginosa* (Ashokkumar, 2014). Lipid content is a key criterion for choosing oleaginous

228 microalga species, and the basal lipid content of microalga are usually limited to not higher than
229 20% or 30% DCW under standard conditions (Hu et al., 2008). The lipid content reported in
230 literature for most microalga was very variable and dependent on the environmental and
231 cultivation conditions. For example, when the microalga cells became old or were exposed to
232 stress conditions, an extraordinary increment in the lipid content could be observed (Hu et al.,
233 2008).

234 The multi criterion decision analysis was performed based on the growth kinetics to select
235 the prominent strains among the fourteen tested cyanobacterial strains for FAME analysis (Fig.
236 2). PROMETHEE displays the decision axis towards the *Calothrix* sp. MBDU 701 which was
237 the most promising strains in the tested parameters viz. biomass and lipid productivity, lipid
238 content. Though *Nostoc spongiaeforme* MBDU 704 showed high lipid productivity, it was
239 averaged among others in biomass productivity. *Calothrix* sp. MBDU 701, *Scytonema bohneri*
240 MBDU 104 and *Calothrix* sp. MBDU 901 were the promising strains in these three parameters
241 analysed and were located along with decision axis. The criteria of lipid productivity and lipid
242 content were directed adjacent to *Nostoc spongiaeforme* MBDU 704. *Nostoc punctiforme*
243 MBDU 621 was positioned orthogonal to the decision axis. This was due to the fact that it is was
244 good in biomass productivity but least in lipid productivity and lipid content (Fig. 2). The phi
245 score displays the rank of these tested cyanobacterial strains and *Nostoc spongiaeforme* MBDU
246 704 was listed as best since it tops the two of three parameters analysed (Table 1). It was
247 followed by *Nostoc punctiforme* MBDU 621, *Scytonema bohneri* MBDU 104, *Calothrix* sp.
248 MBDU 901 and *Calothrix* sp. MBDU 701. In our previous study, we reported that Biowet
249 Extract (BWE) 10 % and 1 % of *Nostoc spongiaeforme* MBDU 704 and *Nostoc punctiforme*
250 MBDU 621, respectively increased the radicle length of *Pisum sativum* seedlings and ranked at

251 third and first place among other tested cyanobacterial strains (Gayathri et al., 2017). The top
252 listed strains were further analysed by GC to study the FAME yield and biodiesel properties.

253 3.1. Fatty acid composition

254 In addition to screening based on biomass productivity, lipid content and lipid productivity, FA
255 profiles of the selected strains were further examined and are considered important for assessing
256 the quality of the biodiesel produced. The quality depends mainly on the unsaturation ratio
257 because unsaturated fatty acids (UFA) enhance cold-flow properties whereas saturated FAs
258 maintain good oxidative stability (Wu and Miao, 2014). Knothe, (2008) reported that Palmitic
259 (C16:0), stearic (C18:0), oleic (C18:1), and linolenic acid (C18:2) as the most common fatty
260 acids contained in biodiesel. In particular, oils with high oleic acid content have been reported to
261 have a reasonable balance of fuel properties. The fatty acid compositions of selected five strains
262 were listed in Table.2. C16:0 (palmitic acid) was the predominant fatty acid group in all strains
263 and it was high in *Scytonema bohneri* MBDU 104 (37.39%) followed by *Calothrix* sp. MBDU
264 901 (25.34%), *Calothrix* sp. MBDU 701 (23.47%), *Nostoc punctiforme* MBDU 621 (21.84%)
265 and *Nostoc spongiaeforme* MBDU 704 (14.39%). The other fatty acid group C16:1 (palmitoleic
266 acid), C18:0 (stearic acid), C18:1 (oleic acid and elaidic acid) were also important for good
267 quality of biofuel since these FAs provide a good balance between cold flow property and
268 oxidative stability (Hoekman et al., 2012). While comparing these groups of fatty acids, C16:1
269 was not detected in *Scytonema bohneri* MBDU 104 and was low in *Calothrix* sp. MBDU 901
270 (1.46%). Whereas, *Calothrix* sp. MBDU 701 showed 22.22% of C16:1, 3.75% of C18:1, 3.93%
271 of C18: 2 (linoleic and linoleaidic acid) and 3.15% of C18:3 (α and γ linolenic acid) fatty acids.
272 The other strains *Nostoc punctiforme* MBDU 621 and *Nostoc spongiaeforme* MBDU 704
273 showed 6.13 % and 5.34% respectively of C16:1.

274 The cyanobacterial strains having the high amount of C16:0 fatty acid showed minimum
275 quantity or absence of other fatty acid group like C18:0, C18:1, C18:2 and C18:3 (Fig. 3).
276 Though dominance of C16:0 makes good feedstock for biodiesel production, presence of C16:1
277 and C18:1 also most common and suitable for biodiesel production. C18:0 was high in *Nostoc*
278 *spongiaeforme* MBDU 704 (10.93%) and *Scytonema bohneri* MBDU 104 (4.8%) while the other
279 strains have limited amount of C18:0 fatty acid. The ratio of C18:1 and C18:2 were not
280 significantly varied among the tested five strains and the high quantity was shown in *Nostoc*
281 *punctiforme* MBDU 621 (7.22%). C18:3 was detected only in *Nostoc punctiforme* MBDU 621
282 (2.25%) and *Calothrix* sp. MBDU 701 (3.15%). The saturated (SFA), monounsaturated (MUFA)
283 and polyunsaturated fatty acids (PUFA) contents of tested cyanobacterial strains were
284 represented in Fig. 4. All the strains showed high amount of SFAs which varied from 36.92% to
285 73.31%; compared to MUFAs (12.18 to 32.79%) and PUFAs (4.85% to 8.64%). SFAs was high
286 in *Calothrix* sp. MBDU 901 (73.31%), followed by *Scytonema bohneri* MBDU 104 (61.12%),
287 *Nostoc spongiaeforme* MBDU 704 (56.23%), *Calothrix* sp. MBDU 701 (37.13%) and *Nostoc*
288 *punctiforme* MBDU 621 (36.92%). Likewise a higher amount of SFA than unsaturated fatty
289 acids was reported in *Synechocystis* PCC 6803 (Velmurugan and Incharoenskadi, 2016).
290
291 Next to SFAs, MUFAs was high in *Calothrix* sp. MBDU 701 (32.79%), followed by *Nostoc*
292 *punctiforme* MBDU 621 (21.51%), *Nostoc spongiaeforme* MBDU 704 (16.2%), *Calothrix* sp.
293 MBDU 901 (15.64) and *Scytonema bohneri* MBDU 104 (12.18%). When the proportion of
294 polyunsaturated FAs (PUFAs) increased, the biodiesel can be easily oxidized and thus reducing
295 the overall CN. Therefore, mono-unsaturated FAs (MUFAs) such as C18:1, which is dominant in
296 high quality feed stock such as canola oil, is generally important and preferred compared to

297 saturated FAs or PUFAs for increasing the quality of biodiesel because they provide balance
298 between cold flow, oxidative stability and combustion properties (Knothe, 2014).

299 *3.2. Biochemical composition*

300 Biochemical composition was also evaluated in terms of total and reducing sugar, protein in
301 dried biomass (Fig. 5). Carbohydrates are the major products derived from photosynthesis and
302 the carbon fixation metabolism (i.e., the Calvin cycle) (Ho et al., 2011). These carbohydrates are
303 either accumulated as starch, or component of cell walls as cellulose, pectin, and sulfated
304 polysaccharides. In microalgae, the composition and metabolism of carbohydrates differ
305 significantly from species to species (Rismani-Yazdi et al., 2011) and it is of great importance in
306 biofuel production with high carbohydrate productivity. The polysaccharides, mainly comprising
307 of cellulose, can be hydrolyzed to obtain reducing sugars for further application in bioethanol
308 fermentation (Sun and Cheng, 2002). A number of studies (Ho et al., 2012; Siaux et al., 2011)
309 have demonstrated that nitrogen-depletion leads to a sharp increase in the lipid or carbohydrate
310 content of microalgae, because this forces them to transform protein or peptides to lipids or
311 carbohydrates. Carbohydrate content was in the range of 0.005-0.072 mg/mL as reducing sugar,
312 0.036 (Fig. 5c) - 0.816 mg/mL as total sugars (Fig. 5b).

313 Protein was in the range of 0.08-0.344 mg/%dwt (Fig.5a) within the limit of already reported
314 literature that ranged between 11.1% and 19.1% (DW) as low quantity which is favorable to
315 microalgal biofuel production since the high protein content means a high proportion of nitrogen
316 in the bio-oil produced.

317

318 *3.3. Fuel properties*

319 The analysis of fatty acid composition can provide useful information to determine the quality of
320 biodiesel parameters like IV, SV, CN, CFPP, LCSF, CP, PP, DU, APE, BAPE, viscosity and
321 density (Table.3). The difference in iodine values are related to fatty acid composition. The
322 European standard defines a maximum value of $120 \text{ g I}_2 \text{ 100 g}^{-1}$, which may be necessary
323 because heating higher unsaturated fatty acids results in the polymerization of glycerides, which
324 leads to the formation of deposits or to the deterioration of the lubricating oil and it may increase
325 due to double bonds in the FA. So limited unsaturated fatty acids may decrease the iodine
326 number (Francisco et al., 2010). In this study, all tested strains showed low iodine value than the
327 maximum accepted standards and proved them as a good candidate for biofuel synthesis.
328 Biodiesel standards did not specify the limit of Saponification value (SV). Table 3 shows the SV
329 of tested cyanobacterial strains and it was in the range of 145.32–217.52. Our results were in
330 consistence with the already reported SV range of 203.18-214.38 (Mandotra et al., 2016).

331 During cold climate, CP and PP are considered important for fuel quality. The CP is the
332 temperature at which a cloud of wax crystals first appear when the fuel is cooled, whereas the PP
333 is the temperature at which the wax formed fuel can flow. Higher proportions of SFAs indicate
334 the higher PP of biodiesel, usually biodiesel has higher CP and PP than diesel fuel (Torres-
335 Jimenez et al., 2011). Biodiesel fuels derived from fats or oils with significant amounts of
336 saturated fatty compounds will display higher CPs and PPs. ASTM D6751 specified CP range of
337 -3 to 12°C and PP of -15 to 20°C . The tested five strains exhibited CP values ranged between
338 2.58 to 17.4°C and PP values of -4.01 to 9.1°C that corroborated with the standard. The CP of
339 *Nostoc punctiforme* MBDU 621 (17.4) and *Scytonema bohneri* MBDU 104 (14.6) was exceeding
340 the standard while the PP of all strains were within the standard limit.

341 Higher CN improves the combustion properties of fuel and easier engine start-up, less
342 occurrence of knocking and low nitrous oxide emission (Arias- Peñarands et al., 2013). Fatty
343 acid profile with higher SFA and MUFA content has higher value of CN. The minimum value of
344 CN specified by EN 14214 and IS 15607 was 51, whereas, in ASTM D6751-08 it was 47
345 (Mandotra et al., 2014). All the tested cyanobacterial strains showed the CN in the range of 65.95
346 to 75.71.

347 Degree of unsaturation (DU) influences the oxidative stability of biodiesel and it is the
348 sum of the masses of MUFA and PUFA (Francisco et al., 2010). The DU was in the range of
349 25.18- 49.07. The DU of *Scenedesmus abundans* at various culture conditions was shown to be
350 in the range of 26.57- 110.04 and was already proven to have good biodiesel properties.
351 The CFPP, which indicates the flow performance of biodiesel at low temperature, is related to
352 the amounts of unsaturated fatty acid in biodiesel (Kwak et al., 2016). All the tested strains met
353 the standard values except *Calothrix* sp. MBDU 901. LCSF is a critical parameter for oxidative
354 stability and determining the cold response of biodiesel. There was no specification for LCSF in
355 the standards and the highest LCSF value was recorded in *Nostoc spongiaeforme* MBDU 704
356 (25.93).

357 The APE and BAPE value in FAME are significant in predicting oxidation stability of the
358 biodiesel (Knothe, 2012). The tested isolates showed APE and BPE range of 11.8-24.9 and 5.8-
359 14.74, respectively. Kinematic viscosity (ν) is the resistance of liquid to flow and depends on the
360 thickness of the oil. The higher viscosity caused insufficient fuel atomization leading to the
361 formation of soot occurs and gets deposited in engine deposits (Shu et al., 2007) while lower
362 viscosity is easier to pump and achieve final droplets to injector (Refaat, 2009). Therefore
363 appropriate kinematic viscosity (ν) of biodiesel ensures adequate fuel supply at different

364 operating temperatures (Ramirez- Veruzco et al., 2012). The ASTM 6751-02, EN 14214, IS
365 15607 has set kinematic viscosity limits to 1.9-6.0 mm² s⁻¹, 3.5-5.0 mm² s⁻¹ and 2.5-6.0 mm² s⁻¹.
366 The range of all strains was between 2.53-4.46 mm² s⁻¹ and meeting the mentioned standards.

367 The fuel injection system supplies fuel by volume not by mass which means denser
368 biodiesel will be injected with greater mass in to the combustion chamber consequently affecting
369 the stoicheometric ratio of air and fuel (Ng et al., 2012). Therefore, density (ρ), for which a
370 standard value has been set at 0.86–0.90 g cm⁻³ according to EN 14214, ASTM D6751-02 and IS
371 15607 is another important parameter for biodiesel quality. FAME profile-derived ρ -values of
372 tested cyanobacterial strains were within this range (0.88).

373 Although, HHV not specified in either ASTM D6751 or EN 14214, heat of combustion
374 impacts fuel efficiency and consumption. In addition, the European heating oil standard, EN
375 14213, specifies that the energy content of FAMEs must be at or above 35 MJ kg⁻¹ (Knothe
376 2010). The HHVs for all samples were relatively similar, with values ranging from 40.15 to
377 42.93 MJ kg⁻¹.

378 Oxidation stability (OS) is the resistance of fuel degradation due to oxidation during
379 long-term storage. Biodiesels show less oxidative stability compared with petroleum diesel due
380 to their different chemical composition, and this is one of the major issues that limits the wide
381 spread use of biodiesel as a fuel in automobile engines. OS was high in *Nostoc spongiaeforme*
382 MBDU 704 (7.67), while all other cyanobacterial strain met the EN 14214 standard.

383 The flash point (FP) is the lowest temperature at which the fuel will begin to vaporize to
384 form an ignitable mixture when it comes in contacts with the air. Australian and European
385 biodiesel specification required flash point temperature of at least 120 °C, whereas in the US the
386 minimum requirement level is 93 °C. Tested cyanobacterial strains showed FP temperature in the

387 range of 184.21 and 199.22°C that was higher than the specified biodiesel standards (Jahirul,
388 2015).

389 3.4. Preference ranking of cyanobacterial strains

390 To produce a profitable biodiesel over diesel fuel, cyanobacteria should have suitable chemical
391 content to establish the concurrence with various biodiesel standards. To figure out the
392 suitability, 15 fuel properties IV, SV, CP, PP, CN, DU, LCSF, CFPP, APE, BAPE, viscosity,
393 density, HHV, SFA, MUFA, PUFA, OS and FP was taken as multiple criteria and analyzed
394 through PROMETHEE-GAIA since it provides logical decision towards the solution compared
395 to other tools (Islam et al., 2013) (Fig. 6a). In GAIA plane, the criteria near to ($\pm 45^\circ$) were
396 correlated, while those in the other side (135° – 225°) were not related and those in orthogonal
397 have no or less impact (Espinasse et al., 1997). For example, *Nostoc spongiaeforme* MBDU 704
398 was correlated since it positioned along with decision axis. The preference function was set to
399 maximum or minimum (lower/higher values preferred for quality based biodiesel) which
400 influenced the orientation of criteria.

401 The direction and length of the criteria influence the decision axis (Islam et al., 2013). For
402 example parameters like OS, viscosity, PUFA, HHV, FP, IV have little effect on the decision
403 vector. The decision axis pointed *Nostoc spongiaeforme* MBDU 704 as best since it was located
404 along with decision axis and *Nostoc punctiforme* MBDU 621 as second since it located adjacent
405 to decision axis and *Calothrix* sp. MBDU 701 followed by other strains (Fig. 6a). Fig. 6b.
406 showed the overall ranking of cyanobacterial strains based on the fuel properties. The Phi value
407 is the net flow score that could be negative or positive depending upon the angular distance from
408 the decision vector and the distance from the centre (Jahirul et al., 2015). Based on the phi score,
409 *Nostoc spongiaeforme* MBDU 704 was the most suitable strain in parameters like PP, CP, OS,

410 viscosity and density. *Nostoc punctiforme* MBDU 621 was preferred in CFPP, MUFA, HHV,
411 PUFA and CFPP. These two strains lie closer to decision axis and met most of the criteria than
412 the other strains tested. The GAIA plane from the analysis has a quality level of 88.5% which is
413 reliable as it was above 70% quality significance level (Ahmad et al., 2015).

414 **4. Conclusion**

415 Here, we report on the ability of heterocystous cyanobacterial strains for biodiesel production.
416 The method of robust strain selection based on FAME profiling and fuel quality parameters
417 using PROMETHEE-GAIA analysis were reported. Based on our study, *Nostoc spongiaeforme*
418 MBDU 704 and *Nostoc punctiforme* MBDU 621 were selected as the promising strains for
419 biodiesel production. These strains were already shown to produce plant growth promoting
420 substances in our earlier work. The cost of biodiesel can greatly be reduced if co-products of
421 commercial value are looked for. Our study highlights this important aspect of multi-potency of
422 cyanobacterial strains for future commercial utilization.

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433 **References**

- 434 1. Agarwal, M., Singh, K., Chaurasia, S., 2010. Prediction of biodiesel properties from fatty
435 acid composition using linear regression and ANN techniques. *Indian Chem. Eng.* 52 (4),
436 347–361.
- 437 2. Ahmad, F.B., Zhang, Z., Doherty, W.O. and O’Hara, I.M., 2015. A multi-criteria analysis
438 approach for ranking and selection of microorganisms for the production of oils for
439 biodiesel production. *Bioresour. Technol.* 190, 264-273.
- 440 3. Anahas, A.M.P., Muralitharan, G., 2015. Isolation and screening of heterocystous
441 cyanobacterial strains for biodiesel production by evaluating the fuel properties from
442 fatty acid methyl ester (FAME) profiles. *Bioresour. Technol.* 184, 9-17.
- 443 4. Angermayr, S.A., Rovira, A.G., Hellingwerf, K.J., 2015. Metabolic engineering of
444 cyanobacteria for the synthesis of commodity products. *Trends Biotechnol.* 33, 352–361.
- 445 5. Ashokkumar, V., Agila, E., Salam, Z., Ponraj, M., Din, M.F.M. and Ani, F.N., 2014. A
446 study on large scale cultivation of *Microcystis aeruginosa* under open raceway pond at
447 semi-continuous mode for biodiesel production. *Bioresour. Technol.* 172, 186-193.
- 448 6. Arias-Peñarands, M.T., Cristiani-Urbina, E., Montes-Horcasitas, C.M., Esparza-Garcia,
449 F., Torzillo, G., Cañizares-Villanueva, R.O., 2013. *Scenedesmus incrassatulus* CLHE-
450 Si01: a potential source of renewable lipid for high quality biodiesel production.
451 *Bioresour. Technol.* 140, 158–164.
- 452 7. Brans, J. P., Mareschal, B., 2005. PROMETHEE methods, multiple criteria decision
453 analysis: state of the art surveys, 163–186.
- 454 8. Cate, J.H., Ball, A.S., 2016. Editorial overview: Energy biotechnology. *Curr. Opin.*
455 *Biotechnol.* 38, v- vii.

- 456 9. Chuck, C.J., Wagner, J.L., Jenkins, R.W., 2015. Biofuels from microalgae, in: Letcher, T.
457 M., Scott, J.L., Patterson, D.A. (Eds.), Chemical Processes for a Sustainable Future.
458 Royal Society of Chemistry, Cambridge, pp. 425–442.
- 459 10. Espinasse, B., Picolet, G., Chouraqui, E., 1997. Negotiation support systems: A multi-
460 criteria and multi-agent approach. Eur. J. Oper. Res. 103(2), 389-409.
- 461 11. Folch, J., Lees, M., Sloan-Stanley, G.H., 1957. A simple method for the isolation and
462 purification of total lipids from animal tissue. J. Biol. Chem. 226, 497–509.
- 463 12. Francisco, É.C., Neves, D.B., Jacob-Lopes, E., Franco, T.T., 2010. Microalgae as
464 feedstock for biodiesel production: carbon dioxide sequestration, lipid production and
465 biofuel quality. J. Chem. Technol. Biotechnol. 85, 395–403.
- 466 13. Gayathri, M., Shunmugam, S., Thajuddin, N., Muralitaran, G., 2017. Phytohormones and
467 free volatile fatty acids from cyanobacterial biomass wet extract (BWE) elicit plant
468 growth promotion. Algal Res. 26, 56-64.
- 469 14. Gerardo, M.L., Van Den Hende, S., Vervaeren, H., Coward, T., Skill, S.C., 2015.
470 Harvesting of microalgae within a biorefinery approach: a review of the developments
471 and case studies from pilot-plants. Algal Res. 11, 248-262.
- 472 15. Gonçalves, A.L., Pires, J.C., Simões, M., 2016. Biotechnological potential of
473 *Synechocystis salina* co-cultures with selected microalgae and cyanobacteria: nutrients
474 removal, biomass and lipid production. Bioresour. Technol. 200, 279-286.
- 475 16. Gravitis, J., 2008. Biorefinery: biomaterials and bioenergy from photosynthesis, within
476 zero emission framework, in: Sustainable Energy Production and Consumption. Springer,
477 pp. 327–337.

- 478 17. Hedge, J.E., Hofreiter, B.T., 1962. in: Whistler R.L., Be Miller, J.N. (Eds.), Carbohydrate
479 Chemistry. Academic Press, New York, 17.
- 480 18. Ho, S.H., Chen, C.Y., Lee, D.J., Chang, J.S., 2011. Perspectives on microalgal CO₂-
481 emission mitigation systems – a review. *Biotechnol. Adv.* 29, 189–198.
- 482 19. Ho, S.H., Chen, Pereira C.Y., Chang, J.S., 2012. Effect of light intensity and nitrogen
483 starvation on CO₂ fixation and lipid/carbohydrate production of an indigenous microalga
484 *Scenedesmus obliquus* CNW-N. *Bioresour. Technol.* 244–252.
- 485 20. Hoekman, S.K., Broch, A., Robbins, C., Ceniceros, E., Natarajan, M., 2012. Review of
486 biodiesel composition, properties, and specifications. *Renew. Sustain. Energy Rev.* 16,
487 143–169.
- 488 21. Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., et al., 2008. Microalgal
489 triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.*
490 54, 621–639.
- 491 22. Indarti, E., Majid, M.I.A., Hashim, R. and Chong, A., 2005. Direct FAME synthesis for
492 rapid total lipid analysis from fish oil and cod liver oil. *J. Food Comp. Anal.* 18(2), 161-
493 170.
- 494 23. Islam, M.A., Magnusson, M., Brown, R.J., Ayoko, G.A., Nabi, M.N., Heimann, K., 2013.
495 Microalgal species selection for biodiesel production based on fuel properties derived
496 from fatty acid profiles. *Energies*, 6(11), 5676-5702.
- 497 24. Jahirul, M.I., Brown, R.J., Senadeera, W., Ashwath, N., Rasul, M.G., Rahman, M.M.,
498 Hossain, F.M., Moghaddam, L., Islam, M.A. O’Hara, I.M., 2015. Physio-chemical
499 assessment of beauty leaf (*Calophyllum inophyllum*) as second-generation biodiesel
500 feedstock. *Energy Reports.* 1, 204-215.

- 501 25. Knothe, G., 2008. “Designer” biodiesel: optimizing fatty ester composition to improve
502 fuel properties. *Energy Fuels* 22, 1358–1364.
- 503 26. Knothe, G., Krahl, J., Van Gerpen, J., 2010. *The biodiesel handbook*, second ed.
504 Champaign (IL) AOCS Press.
- 505 27. Knothe, G., 2012. Fuel properties of highly polyunsaturated fatty acid methyl esters,
506 prediction of fuel properties of algal biodiesel. *Energy Fuels*. 26, 5265-5273.
- 507 28. Knothe, G., 2014. A comprehensive evaluation of the cetane numbers of fatty acid
508 methyl esters. *Fuel*. 119, 6–13.
- 509 29. Kwak, H.S., Kim, J.Y.H., Woo, H.M., Jin, E., Min, B.K., Sim, S.J., 2016. Synergistic
510 effect of multiple stress conditions for improving microalgal lipid production. *Algal Res.*
511 19, 215-224.
- 512 30. Liu, J., Huang, J., Sun, Z., Zhong, Y., Jiang, Y., Chen, F., 2011b. Differential lipid and
513 fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*:
514 assessment of algal oils for biodiesel production. *Bioresour. Technol.* 102, 106–110.
- 515 31. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement
516 with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- 517 32. Mandotra, S.K., Kumar, P., Suseela, M.R., Nayaka, S., Ramteke, P.W., 2016. Evaluation
518 of fatty acid profile and biodiesel properties of microalga *Scenedesmus abundans* under
519 the influence of phosphorus, pH and light intensities. *Bioresour. Technol.* 201, 222-229.
- 520 33. Mandotra, S.K., Kumar, P., Suseela, M.R., Ramteke, P.W., 2014. Fresh water green
521 microalga *Scenedesmus abundans*: a potential feedstock for high quality biodiesel
522 production. *Bioresour. Technol.* 156, 42–47.

- 523 34. Maurya, R., Paliwal, C., Chokshi, K., Pancha, I., Ghosh, T., Satpati, G.G., Pal, R., Ghosh,
524 A., Mishra, S., 2016. Hydrolysate of lipid extracted microalgal biomass residue: An algal
525 growth promoter and enhancer. *Bioresour. Technol.* 207, 197-204.
- 526 35. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing
527 sugar. *Anal. Chem.* 31, 426–428.
- 528 36. Mohan, S.V., Nikhil, G.N., Chiranjeevi, P., Reddy, C.N., Rohit, M.V., Kumar, A.N.,
529 Sarkar, O., 2016. Waste biorefinery models towards sustainable circular bioeconomy:
530 critical review and future perspectives. *Bioresour. Technol.* 215, 2-12.
- 531 37. Ng, J.H., Ng, H.K., Gan, S., 2012. Characterisation of engine-out responses from a light-
532 duty diesel engine fuelled with palm methyl ester (PME). *Appl. Energy.* 90(1), 58-67.
- 533 38. Ramirez-Verduzco, L.F., Rodriguez-Rodriguez, J.E., Jaramillo-Jacob, A.R., 2012.
534 Predicting cetane number, kinematic viscosity, density and higher heating value of
535 biodiesel from its fatty acid methyl ester composition. *Fuel.* 91,102-111.
536 doi:10.1016/j.fuel.2011.06.070.
- 537 39. Refaat, A.A., 2009. Correlation between the chemical structure of biodiesel and its
538 physical properties. *Int. J. Environ. Sci. Technol.* 6, 677-394.
- 539 40. Rippka, R., Deruells, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic
540 assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen.*
541 *Microbiol.* 111, 1–61.
- 542 41. Rismani-Yazdi, H., Haznedaroglu, B.Z., Bibby, K., Peccia, J., 2011. Transcriptome
543 sequencing and annotation of the microalgae *Dunaliella tertiolecta*: pathway description
544 and gene discovery for production of next-generation biofuels. *BMC Genomics* 12, 148.

- 545 42. Shu, Q., Yang, B., Yang, J. and Qing, S., 2007. Predicting the viscosity of biodiesel fuels
546 based on the mixture topological index method. *Fuel*, 86(12), 1849-1854.
- 547 43. Siaut, M., Cuine, S., Cagnon, C., Fessler, B., Nguyen, M., Carrier, P., Beyly, A., Beisson,
548 F., Triantaphylides, C., Li-Beisson, Y., Peltier, G., 2011. Oil accumulation in the model
549 green alga *Chlamydomonas reinhardtii*: characterization, variability between common
550 laboratory strains and relationship with starch reserves. *BMC Biotechnol.* 11, 7.
- 551 44. Slocombe, S.P., Ross, M., Thomas, N., McNeill, S., Stanley, M.S., 2013. A rapid and
552 general method for measurement of protein in micro-algal biomass. *Bioresour. Technol.*
553 129, 51-57.
- 554 45. Sun, Y., Cheng, J.Y., 2002. Hydrolysis of lignocellulosic materials for ethanol
555 production: a review. *Bioresour. Technol.* 83, 1–11.
- 556 46. Talebi, A.F., Mohtashami, S.K., Tabatabaei, M., Tohidfar, M., Bagheri, A.,
557 Zeinalabedini, M., Mirzaei, H.H., Mirzajanzadeh, M., Shafaroudi, S.M., Bakhtiari, S.,
558 2013. Fatty acids profiling: a selective criterion for screening microalgae strains for
559 biodiesel production. *Algal Res.* 2(3), 258-267.
- 560 47. Torres-Jimenez, E., Jerman, M.S., Gregorc, A., Lisec, I., Dorado, M.P., Kegl, B., 2011.
561 Physical and chemical properties of ethanol–diesel fuel blends. *Fuel*, 90(2), 795-802.
- 562 48. Velmurugan, R., Incharoensakdi, A., 2016. Potential of metal oxides in fractionation of
563 *Synechocystis* sp. PCC 6803 biomass for biofuel production. *Algal Res.* 19, 96-103.
- 564 49. Wang, L.B., Yu, H.Y., He, X.H., Liu, R.Y., 2012. Influence of fatty acid composition of
565 woody biodiesel plants on the fuel properties. *J. Fuel Chem. Technol.* 40(4), 397-404.

566 50. Wu, H., Miao, X., 2014. Biodiesel quality and biochemical changes of microalgae
567 *Chlorella pyrenoidosa* and *Scenedesmus obliquus* in response to nitrate levels. *Bioresour.*
568 *Technol.* 170, 421–427.
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570 **Figure legends**

571 **Fig. 1.** The performance of (a) Lipid productivity (mg/L/day) (b) biomass productivity
572 (mg/L/day) and lipid content (%dwt) of fourteen cyanobacterial strains. The bar diagram
573 represent the biomass productivity and lipid content and line art represent the lipid productivity.
574 Data values are means (\pm SE) of three replicates.

575
576 **Fig. 2.** PROMETHEE- GAIA algorithm showing the (a) fourteen cyanobacterial strains
577 indicated in Fuschia dots, pink lines indicated the biomass prodcuctivity (BP), lipid productivity
578 (LP) and lipid content (LC) as criteria, redline is the decision axis

579
580 **Fig. 3.** The major fatty acid content in five cyanobacterial strains appropriate for suitable
581 biodiesel expressed in percentage. The bar diagram represent the C18:0 (stearic), C18:1 (oleic),
582 C18:2 (linoleic) and C18:3 (linolenic) fatty acids. The line art represent the C16:0 (palmitic)
583 fatty acid.

584
585 **Fig. 4.** Fatty acid of five cyanobacterial strains based on the classes represented in percentage.
586 SFA- saturated fatty acid; PUFA- polyunsaturated fatty acid; MUFA- mono unsaturated fatty
587 acid.

588 **Fig. 5.** Biochemical composition of fourteen cyanobacterial strains (a) protein expressed in dwt
589 % (b) carbohydrate estimation by Anthrone method expressed in mg/ml (c) reducing sugar
590 estimation in DNS method expressed in mg/ml.

591

592 **Fig. 6.** PROMETHEE- GAIA algorithm showing (a) five cyanobacterial strains indicated in
593 Fuschia dots, blue lines indicated the fifteen biodiesel properties along with SFA, PUFA and
594 MUFA as criteria, redline is the decision axis (b) PROMETHEE table displays the phi score of
595 five cyanobacterial strains based on the rank obtained through biodiesel properties.

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