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Lipid Accumulation and Biodiesel Production from Filamentous Cyanobacteria Native to Pakistan

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Abstract: Depleting fossil fuel resources and increasing energy demand have intensified the emphasis on biofuel production cyanobacteria. In this study, 19 cyanobacterial filamentous strains were isolated from various regions of Pakistan, including the northern areas and the University of the Punjab, Lahore. Sudan black, Nile red, and BODIPY staining, together with CLSM, fluorimetry, FTIR, growth in different nitrate concentrations, and GC techniques, were used to confirm and measure the lipid and diesel contents within isolated cyanobacterial filaments. *Oscillatoria* sp. strain CFF-6 showed a significantly higher yield (biodiesel/biomass=35.6%). *Leptolyngbya* sp. strain CFF-18 showed a higher yield (biodiesel/biomass=17.1%) compared to other *Leptolyngbya* strains. FAME (Fatty Acid Methyl Ester) analysis was also performed. *Oscillatoria* are better biodiesel producers in comparison to other filamentous strains.

Key words: biofuels, biodiesel, FAME, lipids, hydrocarbons

1 Introduction

Continuous diminution of petrochemicals (oil, coal and natural gas) will result in the inability to fulfill our energy demands in the future and generate problems globally including Pakistan[1, 2]. Daily, the transportation sector uses gasoline, natural gas and diesel in huge amounts[3, 4]. Because of the current 11.6 million tons per day average global oil consumption, fossil fuel subsurface reserves will run out within a few decades[5, 6]. Coal, natural gas and oil or petroleum are the fossil fuels accomplishing the demands of energy need. There is a need to shift towards biofuels because fossil fuels (nonrenewable energy) are depleting due to overconsumption and limited resources, carbon dioxide production which is responsible for global warming, combustion produces toxic gases injurious to human health, crude oil spillage in water poses serious impacts on aquatic life, highly susceptible to price fluctuations and market manipulation, unsustainable; in short there is nothing green or clean about fossil fuels[7]. Biofuels are non-toxic, S-free, biodegradable, and renewable source status, like other renewable energy sources, therefore can be seen as an alternative to oil[6]. Biohydrogen, biomethane, bioethanol and biodiesel are alternative forms of energy obtained from living biomass[8, 9].

Long-standing scientific and technical research has proven that the usage of fossil fuels produces significant amounts of carbon dioxide (CO_2) emissions, which play a key role in causing global warming[10]. Biofuels (bioethanol and biodiesel) are the energy obtained from biomass. Biofuels can substitute transport fuel without greater modifications to vehicle engines[11, 12]. Currently, the frequent and comprehensive available forms of biofuels are biodiesel and bioethanol which are worldwide liquid transportation biofuels and promising substitutes of petro-diesel[13, 14]. Hydrogen is inorganic biofuel whereas methane and ethanol are organic biofuels[15]. Third-generation biofuels use microorganisms that contain a high oil mass fraction with yields per unit area that are many folds higher than those with any other living system[16, 17]. The leading features include high oil mass fraction, higher biomass yield per hectare in comparison to plants, very less or negligible input as fertilizers and continuous harvesting[18–20]. Photosynthetic microorganisms transform H_2O and CO_2 to primary (carbohydrates and lignin) and secondary (steroids, terpenoids, terpenes, triacylglycerol etc.) metabolites which are industrially significant biochemicals. They also have restricted nutritional requirements making them easy to grow at industrial levels[21–23].

Chemicals derived from natural oils and fats (oleochemicals) degrade to fatty acids and glycerol by the breakdown of triacylglycerols in microorganisms. During transesterification, the methyl group of triacylglycerols reacts with the

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free fatty acid to form the fatty acid methyl ester (FAME). The major types of oleochemicals are fatty alcohols, fatty acids, fatty amines and methyl esters of fatty acids. Biodiesel consists of lengthy chain fatty acids monoalkyl esters, produced from TAG or triacylglycerols, DAG or diacylglycerides, FFA or free fatty acids and PL or phospholipids, which are used in vehicle engines. Diversity in different types of fatty acids is on the basis of carbon atoms number, alignment of double bonds and their position as well as the length of carbon chains[24]. The production cost is the major hindrance in the industrialization and commercialization of biodiesel. Microalgae is considered as better feedstock for biodiesel, biohydrogen, biomethane and bioethanol production[25–27]. They are helpful in the mitigation of carbon dioxide release, high photosynthetic conversion efficiency and the growth rate is very high (20-30 times faster than energy crops)[28–30]. Compared with conventional unicellular oleaginous microalgae, filamentous microalgae *Tribonema* sp. is richer in lipid and carbohydrate contents and lower protein content, thus, this study explores the suitability of *Tribonema* sp. The total lipid of *Tribonema* extracted by chloroform–methanol was about 48.7%. Biodiesel physical and chemical fuel properties are determined by the ester moiety and profile of fatty acids[31]. An equilibrium will develop among economic improvement, environment and agricultural domain through the use of biodiesel in practice. Biodiesel is harmless, renewable, compatible, safe in handling, has low emission profiles, has higher flash point and a natural lubricant. It can be used in two forms, either as a pure form of diesel or in a blended form with any fossil fuel based diesel[32–35]. The main objective of the present research was to identify indigenous hyper lipid-producing cyanobacteria strains as significant tools for production of biodiesel.

2 Methods

2.1 Sampling

Different geographically distributed unexplored areas of Pakistan were selected for the collection of samples as shown in Fig. 1[36]. Water and soil samples were collected in contamination free collection bag with sterile spatula and transferred to lab under appropriate temperature conditions (4℃). The samples were subjected to isolation of filamentous cyanobacterial strains.

2.2 Sample enrichment

BG11 media comprises 1.5 g/L $NaNO_3$[37] as a nitrogen source, and its specific chemical composition creates an optimal environment for cyanobacterial growth. This media provides essential nitrogen, phosphorous, and trace elements necessary for the thriving of cyanobacteria. One mL of water sample or 1 g soil sample was directly suspended in autoclaved BG11 medium followed by incubation at 26 ± 2℃ under 16:8 hour alternative light (200 $\mu Em^{-2}\ s^{-1}$) and dark period for 15 to 20 days. This whole procedure was repeated three times.

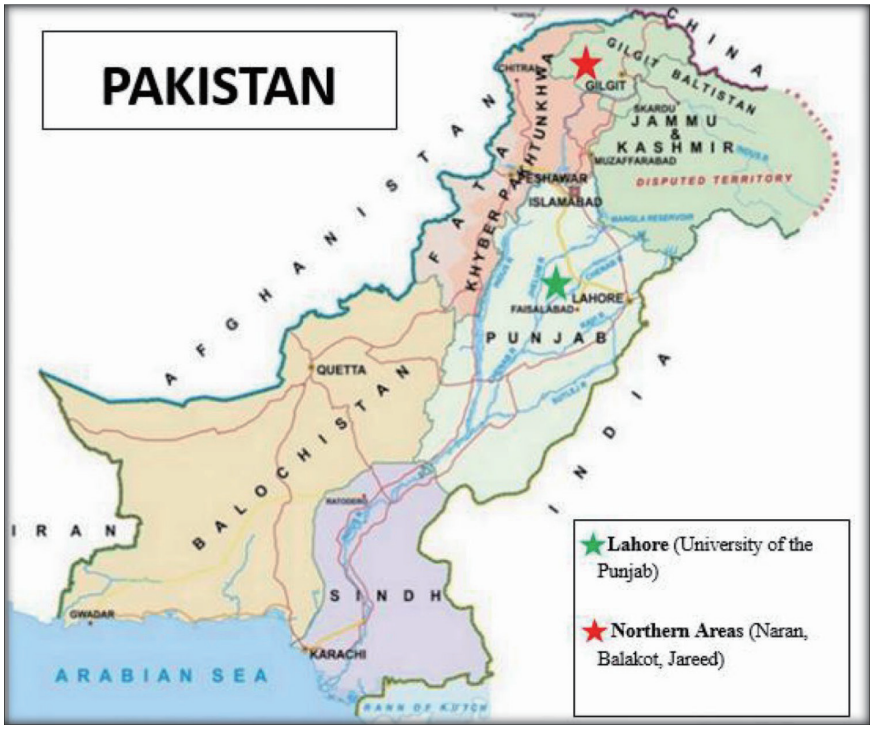

Fig. 1 Geographical representation of sampling sites (Survey of Pakistan, 2016)[67].

2.3 Isolation and purification

With the help of sterile forceps, appropriate amount of inoculum was transferred from incubated liquid BG11 media to BG11 agar plates. Plates were incubated under the same conditions for two weeks and then examined under a microscope. The area showing single filament was marked, cut with a sterile blade and further transferred onto new plate. This process was repeated for 5-6 times until pure monoculture of cyanobacteria was obtained.

2.4 Characterization

2.4.1 Morphological

Compound microscope (OLYMPUS, CX21) and stereo microscope (SWIFT, SM 80, Japan) were used for morphological study of cyanobacteria. Different physical parameters such as shape, length and width of filaments, color and either motile or non-motile were studied.

2.4.2 Genetic identification

The exact taxonomic status of cyanobacterial isolates was determined by 16S rRNA gene sequencing. Purified strains were sent to Macrogen Inc Korea for sequencing and genetic identification. All the obtained sequences were edited and assembled by using CLC Workbench software. Final 16S rRNA gene sequences were related with NCBI sequence database (GenBank) through Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST).

2.5 Localization of lipids

2.5.1 Sudan Black B staining

Sudan black B, chemically also known as ((2,2-dimethyl-1,3-dihydroperimidin-6-yl)-(4-phenylazo-1-naphthyl) diazene), is a lipophilic diazo dye. Smears of cyanobacterial isolates were flooded with 2-3 mL of Sudan black solution (CAS No: (4197-25-5)/ 199664 SIGMA-ALDRICH) (0.3% Sudan black in 70% ethanol) and kept undisturbed at room temperature for 5 to 15 minutes. The stains were drained out and then washed with water and kept for air drying. Subsequently, slides were observed under a compound microscope[38].

2.5.2 Confocal Laser Scanning Microscopy

Two Lipid Fluorescent dyes i.e., Nile Red (9-(Diethylamino)-5H-benzo[α]phenoxazin-5-one) and BODIPY 493/503 were used as tools to observe the cyanobacterial intracellular as well as extracellular lipids. Nile red is useful for the detection of neutral lipids or triacylglycerols (TAG). In 1 mL Nile red working solution (excitation wavelength = 493 nm and emission wavelength = 517 nm) or 1 mL BODIPY dye solution (excitation wavelength = 494 nm and emission wavelength = 518 nm) few filaments of cyanobacterial strains were incubated in the dark for 20 minutes and then observed under confocal laser scanning microscope (Leica True Confocal Scanning - Spectral Photometric 5 - Model upright DM 6000S)[39–43].

2.6 Optimization of lipids accumulation by strains (Fluorimetry)

Microplate reader (Plate CHAMELEONTM V425-106 Multilabel Counter; Software, Hidex CHAMELEON MikroWin 2000, v. 4.43) was used to monitor lipids quantity in cyanobacterial filaments by following the phenomena of fluorescence[44]. Under stress, the amount of oil can surpass 80% by weight of dry biomass. Microwin 2000 software was used for the analysis and quantification of intensity of fluorescence. Nile red dye (excitation wavelength of 485 nm and emission wavelength of 590 nm) and BODIPY dyes were used for the detection of fatty acids with or without stress.

2.7 Lipid extraction and transesterification

Two to three weeks incubated cyanobacterial cultures were centrifuged and biomass was transferred to vials. The samples were dried overnight in a 40℃ under vacuum (5301 VacuFuge, Centrifugal Vacuum Concentrator). After drying and cooling, the weight of the moisture free cyanobacterial sample was calculated.

For transesterification, 25 μL prepared methyl tridecanoate as internal standard (10 mg/mL methyl tridecanoate (C13:0ME) in *n*-hexane), 200 μL Chloroform:Methanol (2:1) and 300 μL of 0.6 M HCl:Methanol was added in a GC vial containing 1 g of cyanobacterial dried biomass. All the components were vigorously mixed and placed for heating at 85℃ ± 3℃ in a preheating digital dry block (Fisher Scientific Dry Block Incubator Fischer Scientific Dry Bath Incubator)[45, 46].

2.8 Fatty Acid Methyl Esters (FAME) analysis

One mL HPLC grade *n*-hexane was added to each cooled vial after removing from dry heating block and kept for one hour to allow the phases (lipids and non-lipids layers/glycerin) to separate. For the estimation of high lipid content (e.g., late harvesting), a new set of GC vials were labeled and added with 450 μL *n*-hexane and 50 μL of upper phase (lipid) of sample (from the previous step). FAME standards (C8:0-C24:0) neat mix, SUPELCO F.A.M.E. Mix, C8-C24, 18918 lipid standards) along with the internal standard (methyl tridecanoate (C13:0ME), T0627 SIGMA) was also prepared for the preparation of calibration curve[47]. Lipid samples were analyzed and measured by gas chromatography (GC) (PerkinElmer GC 580).

2.9 FTIR analysis

FTIR (Bruker Alpha-P Fourier Transform Infrared Spectrometer (Bruker Optics GmbH, Germany) was used to analyze the functional groups present in the crude biodiesel sample after the transesterification process. A small drop of diesel sample was placed on KBr pellet and analyzed for different sulfate, carboxyl and hydroxyl groups in the sample.

2.10 Diesel production from consortium and isolated strains

Mixed cyanobacterial cultures were harvested after centrifugation at 10,000 *g* for 10 min. The biomass was used for the extraction of lipids[48] and converted into biodiesel[49]. The above-mentioned procedure was done in triplicates.

2.11 Effect of nitrate on biodiesel production

Cyanobacterial filaments were grown in BG11 media (1.5 g $NaNO_3$), $BG11_0$ media and BG11 media supplemented by different concentrations of $NaNO_3$ (0.5%, 1%, 1.5% and 2%). Ten selected strains were used to check the effect of $NaNO_3$ concentration on biodiesel production from them[49].

2.12 Field culturing experiments

A modified media, an amalgam of three different types of media, was used for the mass cultivation (10-liter media) of cyanobacteria. For one-liter media, Neem extract and coir pith mixture medium, BG11 medium and soil extract medium were combined in a ratio of 7:2:1. Mass cultivation media was transferred to each container and then strains inoculum was given in each container. The whole setup was incubated for 45 days under that of 15,000 lux at 35-37℃. After 45 days of growth, cultures were harvested for the extraction of lipids from biomass.

3 Results

3.1 Sampling, isolation and purification

The physiological characters of sampling site (Temperature, pH, Latitude and Longitude) were noted and described in Table 1. Filamentous cyanobacterial strains were isolated and purified as *Oscillatoria*, *Leptolyngbya*, *Lyngbya*, *Phormidium* and *Microcoleus*. The accession numbers of representative strains are given as KF157403, KF15740 and KF157406 for *Phormidium* sp., *Oscillatoria* sp., and *Leptolyngbya* sp., respectively. These strains were

Table 1 Representation of sampling site physiological characters.

Sr. No.	Sampling location	Latitude / Longitude	Temperature	pH	Time / Date
Location A	Naran (water sample)	34.91 N / 73.65 E	0℃	8	Sep 29, 2014 / 11:00 am
Location B	Naran (soil sample)	34.91 N / 73.65 E	0℃	8	Sep 29, 2014 / 11:30 am
Location C	PU rice field (soil sample)	31.49 N / 74.29 E	40℃	7.5	Aug 15, 2014 / 10:00 am
Location D	Jareed (water sample)	34.55 N / 73.34 E	2-3℃	8	Sep 27, 2014 / 2 pm
Location E	Balakot (water sample)	34.70 N / 73.55 E	4℃	8	Sep 30, 2014 / 11:00 am
Location F	PU vegetable farm (water sample)	31.49 N / 74.29 E	35℃	7	Oct 18, 2014 / 11:45 am
Location G	PU vegetable farm (soil sample)	31.49 N / 74.29 E	35℃	7.4	Oct 18, 2014 / 11:50 am
Location H	PU rice field (water sample)	31.49 N / 74.29 E	40℃	7.7	Aug 15, 2014 / 10:30 am
Location I	PU rice field (water sample)	31.49 N / 74.29 E	40℃	7.7	Aug 15, 2014 / 10:45 am
Location J	PU water Channel (water sample)	31.49 N / 74.29 E	33℃	8	Oct 18, 2014 / 11:10 am
Location K	PU fish pond (soil sample)	31.49 N / 74.29 E	33℃	8.2	Oct 18, 2014 / 09:45 am
Location L	Balakot (soil sample)	34.70 N / 73.55 E	4℃	8.2	Sep 30, 2014 / 11:45 am

labelled as CFF (cyanobacteria fuel filament).

3.2 Characterization

3.2.1 Morphological characterization

Morphological characters of isolated strains were examined under a compound microscope at 100X, 400X and 1000X magnification as described in Table 2. The subsequent parameters such as the color of trichome, trichome width, sheath width and motility of trichome were observed (Fig. 2).

3.2.2 Genetic identification

Filamentous isolates were purified and morphologically determined, followed by sequencing (Macrogen Inc Korea). Sequences were submitted to GenBank. Results are summarized in Table 3.

3.3 Localization of lipids

3.3.1 Sudan Black staining

The appearance of black color in the trichome shows the presence of lipids within cells after staining with Sudan black dye. Both types of lipids were observed. i.e., larger lipid molecules and small-sized lipid molecules. Their exopolysaccharides (EPS) also showed the presence of lipids thus stained with Sudan black dye and appearing black as shown in Fig. 3. More lipid molecules were observed in the case of *Oscillatoria* spp. (strains CFF-1, CFF-3, CFF-4, CFF-6, CFF-7, CFF-10 and CFF-11) and these lipid molecules were smaller in size. Subsequent higher lipid amount was observed in *Leptolyngbya* spp. (strains CFF-9, CFF-12, CFF-13, CFF-14, CFF-15, CFF-17, CFF-18 and CFF-19). In comparison to *Oscillatoria* spp. and *Leptolyngbya* spp., relatively lower amounts of lipid moieties were observed in *Lyngbya* sp. strain CFF-8, *Phormidium* sp. strain CFF-2, *Phormidium* sp. strain CFF-5 and *Microcoleus* sp. strain CFF-16. The number of lipid molecules were lesser in *Lyngbya* sp. strain CFF-8 but they were larger in size. Secretary lipid molecules were observed in *Leptolyngbya* sp. strain CFF-19.

3.3.2 Confocal Laser Scanning Microscopy (CLSM)

Lipid particles in trichrome were visualized by Nile red dye under a confocal laser scanning microscope. The lipid molecules appeared as shiny spherical molecules and constituted most of the majority of the cell volume having the characteristic yellow color fluorescence as shown in Fig. 4. The lipid fluorescence in all isolated *Oscillatoria* spp. (strains CFF-1, CFF-3, CFF-4, CFF-6, CFF-7, CFF-10 and CFF-11) was three times higher than *Lyngbya* spp. strain CFF-8 and two-fold higher than *Leptolyngbya* spp. (strains CFF-9, CFF-12, CFF-13, CFF-14, CFF-15, CFF-17, CFF-18 and CFF-19). A lesser amount of neutral lipids fluorescence was observed in *Microcoleus* sp. strain CFF-16, *Phormidium* spp. strain CFF-2 and strain CFF-5. The

Table 2 Morphological description of isolated filamentous cyanobacterial strains.

Strain No.	Strain	Trichome features	Sheath thickness	Motility pattern
1.	*Oscillatoria* spp.	Dark green 3.5 μm diameter Unbranched Hormogonia formation	0.5 μm	Oscillatory movement (Average speed=3 μms^{-1})
2.	*Phormidium* spp.	Green 6-7 μm (width) Unbranched Terminal cells pointed Heterocyst absent	0.4 μm	Motile (Average speed=4 μms^{-1})
3.	*Microcoleus* spp.	Dark green Unbranched Untapered ends Cells 6-14 μm (width) Cells 4-6 μm (length)	−	Back and forth movement
4.	*Leptolyngbya* spp.	Green 1-2 μm (width) Cells disc shaped (0.5 × 1.5 μm) Mostly coiled in form of clusters Non-heterocystous	0.25 μm Colorless	−
5.	*Lyngbya* spp.	Brown green Unbranched 1 μm-7.5 μm (diameter) Hormogonia formation	0.5 μm Rigid Mucilaginous	Motile

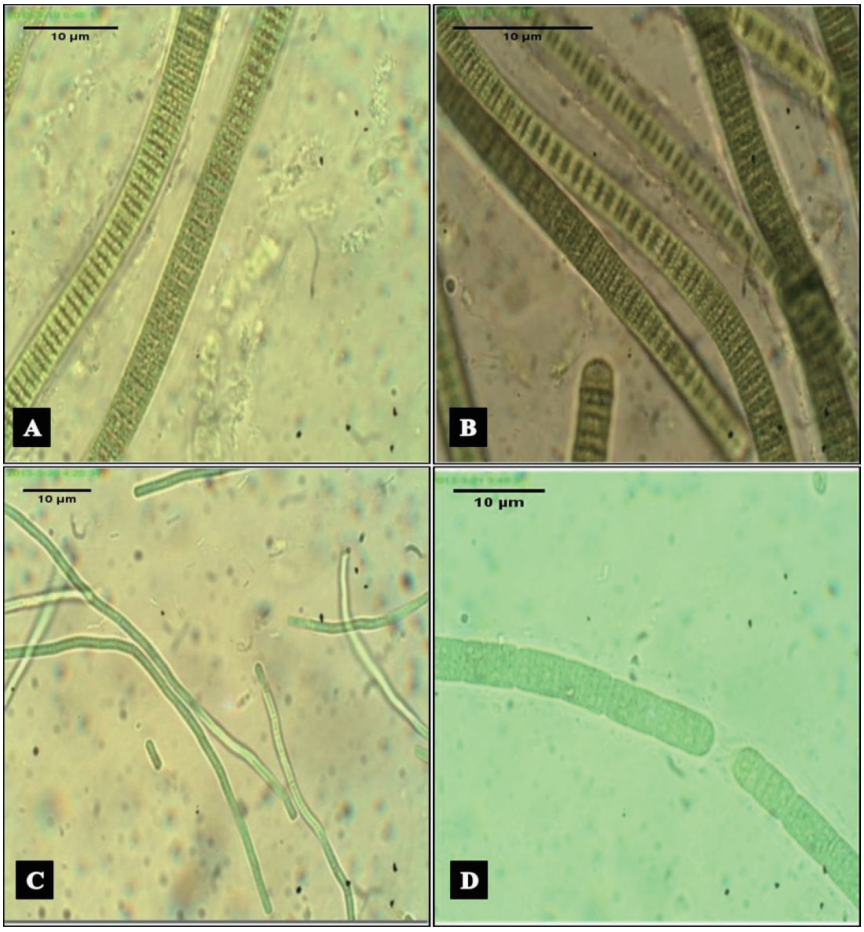

Fig. 2 Photomicrographs of different morphotypes found in environmental samples and of isolated strains. (A = *Oscillatoria* sp. CFF-6, B = *Oscillatoria* sp. CFF-10, C = *Leptolyngbya* sp. CFF-12 and D = *Phormidium* sp. CFF-5).

Table 3 Genetic identification of isolated filamentous cyanobacterial strains.

Sr. No.	Genera	Strains
1.	*Oscillatoria* spp.	CFF-1, CFF-3, CFF-4, CFF-6, CFF-7, CFF-10, CFF-11
2.	*Phormidium* spp.	CFF-2, CFF-5
3.	*Lyngbya* sp.	CFF-8
4.	*Leptolyngbya* spp.	CFF-9, CFF-12, CFF-13, CFF-14, CFF-15, CFF-17, CFF-18, CFF-19
5.	*Microcoleus* sp.	CFF-16

same lipid fluorescence pattern was observed by the aforementioned strains with BODIPY dye. The maximum fluorescence was observed by *Oscillatoria* strains. The lipids were fluoresced as green color shiny molecules by this dye as shown in Fig. 5.

3.4 Optimization of lipid accumulation by strains (Fluorimetry)

In the absence of nitrate, *Microcoleus* sp. strain CFF-16 gave significant fluorescence (17.86 and 17.01) with BODIPY/Acetone (working solution 3 and 4). *Leptolyngbya* sp. strain CFF-15 showed the best fluorescence (15.39) of lipids with working solution 2 of BODIPY/Acetone. *Oscillatoria* sp. strain CFF-7 (16.73) with BODIPY/Acetone (working solution 2 in media 1) and *Microcoleus* sp. strain CFF-16 showed higher fluorescence (16 and 15.88) of lipids with BODIPY/Acetone (working solution 2 and working solution 1 respectively in media[50]. *Leptolyngbya* sp. strain CFF-12 showed significant lipids fluorescence such as 18.23 with BODIPY/DMSO (working solution 2 in $BG11_0$ media). *Oscillatoria* sp. strain CFF-11 showed significant lipid fluorescence (11.06) with Nile Red/DMSO (dilution 4 in $BG11_0$ media).

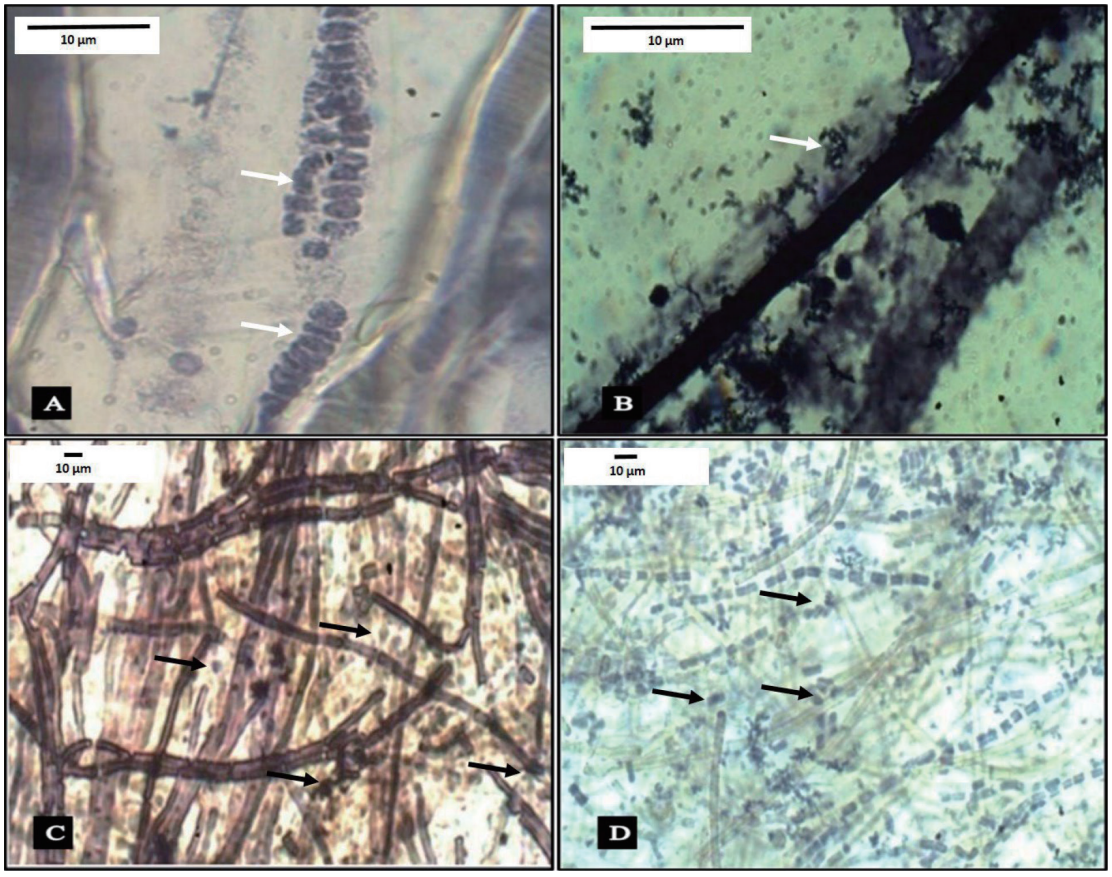

Fig. 3 Sudan Black staining, A = *Oscillatoria* sp. strain CFF-3, B = *Leptolyngbya* sp. strain CFF-19, C = *Lyngbya* sp. strain CFF-8, D = *Microcoleus* sp. strainCFF-16.

3.5 Lipid extraction and transesterification

Among *Oscillatoria* strains, *Oscillatoria* sp. strain CFF-6 showed 35% Diesel/Biomass. Among *Leptolyngbya* strains, *Leptolyngbya* sp. strain CFF-17 and *Leptolyngbya* sp. strain CFF-18 have shown 17% Diesel/Biomass.

3.6 FAME analysis

Fatty acid methyl ester standard, $C_{8:0}$-$C_{24:0}$ neat mix, was used to compare the type of lipids in isolated cyanobacterial strains. Lipids (extracted from isolated strains) detected by gas chromatography as shown in Fig. 6 were methyl octanoate or caprylic acid methyl ester ($C_{8:0}$ = 8%); methyl decanoate or capric acid methyl ester ($C_{10:0}$ = 8%); lauric acid methyl ester ($C_{12:0}$ = 8%); methyl tetradecanoate or myristic acid methyl ester ($C_{14:0}$ = 8%); palmitic acid methyl ester ($C_{16:0}$ = 11%); methyl Palmitoleic acid methyl ester ($C_{16:1}$, *cis*-9 = 5%); methyl octadecanoate or stearic acid methyl ester ($C_{18:0}$ = 8%); *cis*-9-oleic acid methyl ester ($C_{18:1}$, *cis*-9 = 5%); linoleic acid methyl ester ($C_{18:2}$, *cis*-9,12 = 5%); methyl docosanoate or linolenic acid methyl ester ($C_{18:3}$, *cis*-9,12,15 = 5%); arachidic acid methyl ester ($C_{20:0}$ = 8%); behenic acid methyl ester or methyl docosanoate ($C_{22:0}$ = 8%); erucic acid methyl ester ($C_{22:1}$, *cis*-13 = 5%) and lignoceric acid methyl ester ($C_{24:0}$ = 8%) as shown in Table 4. Methyl palmitate, methyl palmitoleate and methyl docosanoate were present in all isolated strains.

3.7 FTIR analysis

Fourier Transform Infrared Red Spectroscopy (FTIR) is a powerful tool for the detection of diverse functional groups at a specific wavelength or wavelength spectrum for lipid analysis. Different major and minor peaks were observed in the filamentous cyanobacterial biodiesel samples between 500 and 5000 cm^{-1} (Fig. 7). FTIR spectrum revealed presence of functional groups such as CH = O among wavelengths of 2720 to 2750 cm^{-1}, COOH in midst of wavelengths 2200 to 2800 cm^{-1}, O = C-O-C = O in between wavelengths of 1740-1770 cm^{-1}, OH (λ = 1300-1500 cm^{-1}), C-O to C-C functional group (λ = 1025-1200 cm^{-1}), aromatic ring (λ = 1440 to 1600 cm^{-1}), C≅N (λ = 2240-2260 cm^{-1}) and $=CH_2$ (λ = 909 or 993 cm^{-1}) in the biodiesel.

3.8 Biodiesel production from consortium and isolated strains

Biodiesel was extracted from the cyanobacteria grown as a consortium in lab conditions at 26 ± 2℃ under 16:8 hours

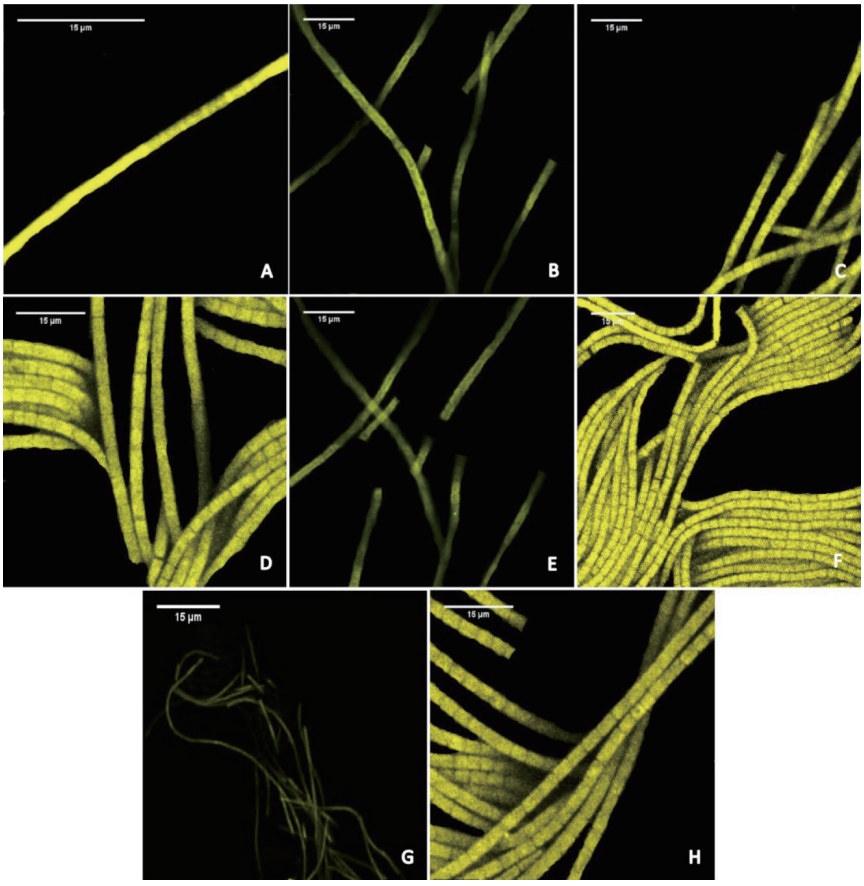

Fig. 4 Confocal microscopy of cyanobacteria lipids (by the staining of Nile red stain) showing yellow color fluorescence (A = *Lyngbya* sp. strain CFF-8, B = *Phormidium* sp. strain CFF-5, C = *Oscillatoria* sp. strain CFF-6, D = *Oscillatoria* sp. strain CFF-10, E = *Microcoleus* sp. strain CFF-16, F = *Oscillatoria* sp. strain CFF-4, G = *Leptolyngbya* sp. strain CFF-12 and H = *Oscillatoria* sp. strain CFF-11).

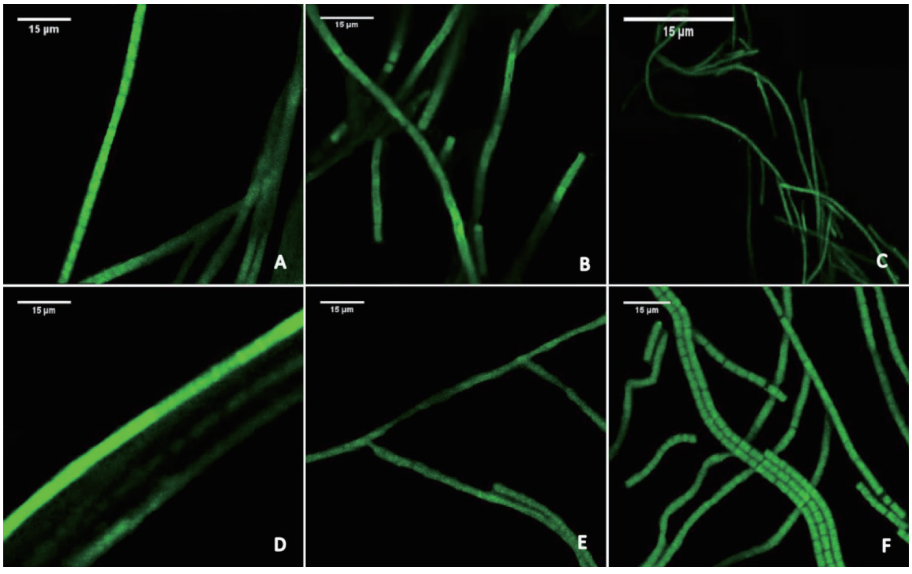

Fig. 5 Confocal microscopy of cyanobacteria lipids (by the staining of BODIPY stain) showing green color fluorescence (A = *Lyngbya* sp. strain CFF-8, B = *Phormidium* sp. strain CFF-5, C = *Leptolyngbya* sp. strain CFF-12, D = *Oscillatoria* sp. strain CFF-6, E = *Microcoleus* sp. strain CFF-16 and F = *Oscillatoria* sp. strain CFF-11).

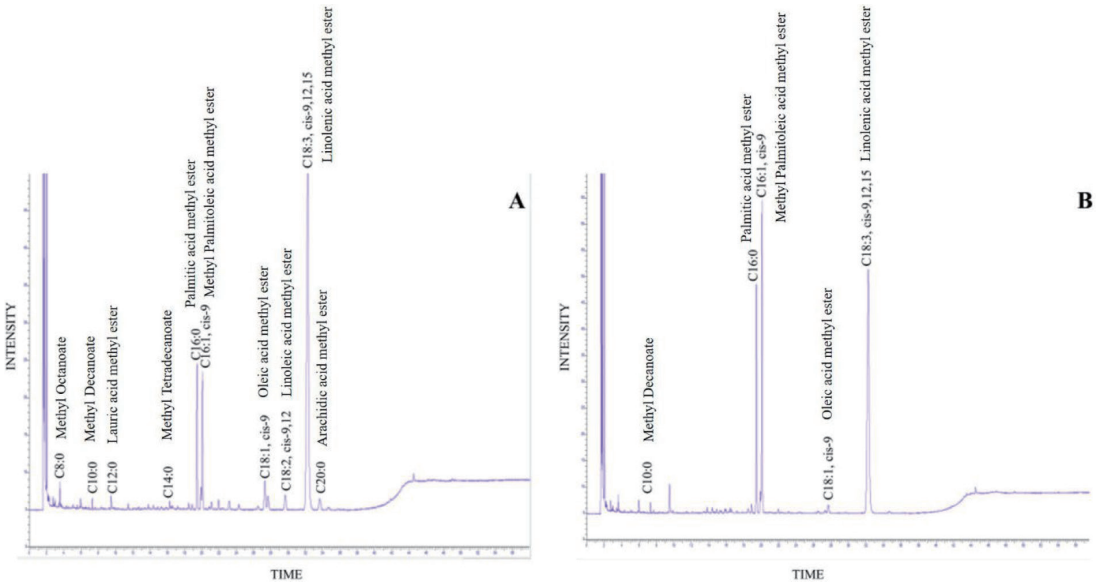

Fig. 6 Gas chromatography showing different types of lipids in form of peaks A: *Leptolyngbya* sp. strain CFF-14, B: *Leptolyngbya* sp. strain CFF-12.

in different light and dark periods (Light intensity; 200 $\mu Em^{-2}\ s^{-1}$) for 2 to 3 weeks. Various biodiesel parameters were as shown in Table 5 along with the amount of biodiesel obtained from each consortium. The significantly higher yield (44.96%, 45.61%, 41.66%, 42.81% and 45.8%) was obtained from consortia B, consortia C, consortia F, consortia H and consortia J, respectively.

Filamentous isolates were grown in BG11 media for the growth of each strain and extraction of lipids. Amount of biodiesel (Table 6) was measured after the extraction of lipids and their conversion into biodiesel was done by the process of transesterification. The maximum yield was observed by *Oscillatoria* sp. strain CFF-6 (35.58%). The greater amount of biomass was obtained by *Microcoleus* sp. strain CFF-16. *Phormidium* sp. strain CFF-2 (almost 8 g/500 mL BG11 media) also showed a higher amount of biomass production but the amount of biodiesel obtained from the abovementioned strain was significantly less (600 µL) than others.

3.9 Effect of nitrate on biodiesel production

Some selected filamentous isolates were grown in BG11 medium containing different $NaNO_3$ concentrations such as $BG11_0$, BG11 media containing various concentrations of nitrate i.e., $BG11_{0.5\%}$, $BG11_{1\%}$, $BG11_{1.5\%}$ and $BG11_{2\%}$ for biodiesel production. Strains were harvested for the extraction of lipids and subsequently transesterification for the production of oil/biomass. The higher percentage yield was obtained in the case of $BG11_{1.5\%}$ as shown in Table 7. The significantly higher yield was obtained by *Oscillatoria* sp. strain CFF-1 (40.82%), *Oscillatoria* sp. strain CFF-6 (47.18%) and *Oscillatoria* sp. strain CFF-7 (41.32%). The whole procedure was conducted three times.

3.10 Field culturing experiment

Some selected isolates were grown in a natural environment during August, September and October. The biomass and amount of diesel were higher in *Oscillatoria* sp. strain CFF-3 (13 mL biodiesel from 35 g biomass) and *Leptolyngbya* sp. strain CFF-18 (14 mL biodiesel from 35 g biomass). The percentage yield of diesel per biomass in *Oscillatoria* sp. strain CFF-6 was significantly higher (48.08%) as shown in Table 8.

4 Discussion

Cyanobacteria are efficient autotrophic prokaryotes known for the production of various kinds of biofuels[51]. The presence of triacylglycerols (building blocks of biodiesel) in cyanobacteria makes them an important source of energy. Fatty acid composition of cyanobacteria is affected by many factors including nutritional factors, environmental factors, cultivation conditions (light intensity, temperature) and different growth phases[9, 52, 53]. Nineteen filamentous cyanobacterial strains were isolated and then purified by growing in sterile BG11 medium under 16:8 hour alternative light and dark period (200 $\mu Em^{-2}\ s^{-1}$ light intensity) at 26 ± 2℃ for 15 to 20 days. Under optimized abiotic conditions, 57% FAME (Fatty acid methyl esters) yield has been observed in *Oscillatoria* sp.

Microscopic analysis was performed for the morphological identification of the strains by compound and stereo microscopes. Based on their morphology i.e., trichome

Table 4 Relative quantity (%) of fatty acids in lipids of isolated cyanobacterial strains.

	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1, *cis*-9	C18:0	C18:1, *cis*-9	C18:2, *cis*-9,12	C18:3, *cis*-9,12,15	C20:0	C22:0	C22:1, *cis*-13	C24:0
CFF-1	0	0	0	0.5	18.2	25.3	0	1.2	0	54.8	0	0	0	0
CFF-2	0	0	0.6	0	15.3	6.5	0	8.8	0.6	68.2	0	0	0	0
CFF-3	0	0	0	0	2.8	1.3	0	0	0	95.9	0	0	0	0
CFF-4	0	0	0	0	33.6	19.9	0	14.6	18.9	12.9	0	0	0	0
CFF-5	0	0.8	0.3	0.3	6.5	9.0	0	0.1	1.0	80.9	0	0	0.8	1.3
CFF-6	0	0	0	0	19.6	28.3	0	2.0	0	50.8	0	0	0	0
CFF-7	0	0.2	0.2	0.1	13.9	18.1	0.2	2.0	1.0	64.5	0	0	0	0
CFF-8	0	0	0	0	11.3	16.6	0	0	0	72.0	0	0	0	0
CFF-9	0	0	0.2	0.1	11.4	14.3	0	1.0	0	73.1	0	0	0	0
CFF-10	0	0	0.2	0	1.63	1.3	0	0	0	96.9	0	0	0	0
CFF-11	0	0	0.6	0.4	51.8	22.8	0	6.6	13.4	4.21	0.2	0	0	0
CFF-12	0	1.6	0	0	34.5	40.3	0	1.5	7.4	14.8	0	0	0	0
CFF-13	0	0	0.3	0.3	18.0	22.7	0	0.1	2.5	56.1	0	0	0	0
CFF-14	0.2	0.3	0.7	0.5	27.1	30.6	0	2.8	0.3	37.2	0.4	0	0	0
CFF-15	0	0.4	0.5	0	12.7	14.4	0	2.2	0	53.5	0.4	0	0	15.9
CFF-16	0	0.4	0.1	0.4	24.5	36.6	0	0.3	0.1	37.4	0	0	0.2	0
CFF-17	0	0	0	0	20.6	25.0	0	2.1	0	52.4	0	0	0	0
CFF-18	0	0.3	0.2	0.4	30.0	41.8	0	3.2	0.1	23.9	0	0	1.0	0
CFF-19	0	0	0	0.4	10.3	4.7	1.0	0.6	6.9	76.2	0	0	0.2	0

Strains: CFF-1 = *Oscillatoria* sp. strain; CFF-2 = *Phormidium* sp. strain; CFF-3 = *Oscillatoria* sp. strain; CFF-4 = *Oscillatoria* sp. strain; CFF-5 = *Phormidium* sp. strain; CFF-6 = *Oscillatoria* sp. strain; CFF-7 = *Oscillatoria* sp. strain; CFF-8 = *Lyngbya* sp. strain; CFF-9 = *Leptolyngbya* sp. strain; CFF-10 = *Oscillatoria* sp. strain; CFF-11 = *Oscillatoria* sp. strain; CFF-12 = *Leptolyngbya* sp. strain; CFF-13 = *Leptolyngbya* sp. strain; CFF-14 = *Leptolyngbya* sp. strain; CFF-15 = *Leptolyngbya* sp. strain; CFF-16 = *Microcoleus* sp. strain; CFF-17 = *Leptolyngbya* sp. strain; CFF-18 = *Leptolyngbya* sp. strain; CFF-19 = *Leptolyngbya* sp. strain.

Fatty Acids: $C_{8:0}$ = methyl octanoate or caprylic acid methyl ester; $C_{10:0}$ = methyl decanoate or capric acid methyl ester; $C_{12:0}$ = lauric acid methyl ester; $C_{14:0}$ = methyl tetradecanoate or myristic acid methyl ester; $C_{16:0}$ = palmitic acid methyl ester; $C_{16:1}$ = methyl Palmitoleic acid methyl ester; $C_{18:0}$ = methyl octadecanoate or stearic acid methyl ester; $C_{18:1}$ = *cis*-9-oleic acid methyl ester; $C_{18:2}$, *cis*-9,12 = linoleic acid methyl ester; $C_{18:3}$, *cis*-9,12,15 = methyl docosanoate or linolenic acid methyl ester; $C_{20:0}$ = arachidic acid methyl ester; $C_{22:0}$ = behenic acid methyl ester or methyl docosanoate; $C_{22:1}$, *cis*-13 = erucic acid methyl ester and $C_{24:0}$ = lignoceric acid methyl ester.

width, sheath width, trichome shape, strains were named accordingly. Sudan black stain is useful for screening hyper lipid-producing cyanobacteria. There is a positive correlation between hyper biodiesel production and the presence of more lipid staining within microorganisms[54]. Both types of lipids were observed. i.e., larger lipid molecules and small-sized lipid molecules. Their exo-polysaccharides also showed the presence of lipids. Their EPS was composed of lipids, thus stained with dye and appearing black. Different intensities in color uptake of Sudan black were dependent on the presence of lipid content in the cyanobacteria trichome[55]. Among all isolated *Oscillatoria* species, strain CFF-6 showed a higher amount of lipids by higher strength of lipid staining. The lipid molecules were detected throughout the trichome. Among the *Leptolyngbya* species, strain CFF-17 showed higher lipids content. A smaller amount of lipids was observed in *Microcoleus* sp. strain CFF-16. Along with the intracellular lipids, lipid par-

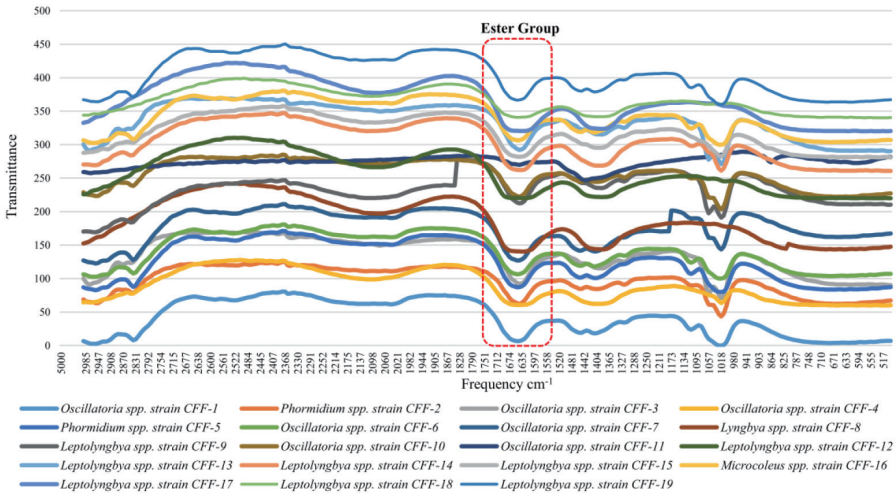

Fig. 7 FTIR spectra of selected filamentous cyanobacterial strains.

Table 5 Production of biodiesel from consortium (in laboratory).

Sample	Wet weight of biomass (g)	Amount of diesel (mL)	Diesel/ Biomass %	Color	Viscosity	Odor
Consortia A	16.1 ± 2.0 (bc)	5.7 ± 1.1 (e,f)	35.6 (c,d,e)	Yellow	✓	✓
Consortia B	15.9 ± 2.0 (bc)	7.1 ± 1.1 (f,g)	45.0 (e,f)	Off White	×	×
Consortia C	12.5 ± 2.31 (ab)	5.7 ± 1.3 (ef)	45.6 (f)	Light Yellow	✓	✓
Consortia D	11.3 ± 2.17 (a)	3.8 ± 1.3 (abc)	34.1 (cd)	Yellow	×	×
Consortia E	11.7 ± 1.07 (ab)	3.4 ± 1.8 (ab)	29.1 (bc)	Yellow	✓	✓
Consortia F	19.2 ± 2.94 (cd)	8 ± 1.7 (g)	41.7 (def)	White	×	×
Consortia G	22.6 ± 1.08 (d)	5.1 ± 1.1 (cde)	22.7 (ab)	Light Yellow	✓	×
Consortia H	19.3 ± 1.08 (cd)	8.3 ± 0.9 (g)	42.8 (def)	Light Yellow	×	✓
Consortia I	19.7 ± 1.43 (cd)	5.5 ± 1.7 (de)	27.5 (abc)	Yellow	✓	×
Consortia J	10 ± 1.50 (a)	4.6 ± 0.9 (bcde)	45.8 (f)	Yellow	×	×
Consortia K	19.3 ± 1.2 (cd)	4 ± 1.2 (abcd)	20.7 (ab)	Yellow	✓	✓
Consortia L	14.52 ± 1.42 (ab)	2.7 ± 1.9 (a)	18.6 (a)	Yellow	×	✓

Mean of 3 values ± Standard deviation, ✓ = presence, × = absence. In each column, figures followed by different letter(s) are significantly different by Duncan's multiple range test (DMRT) ($p < 0.05$).

ticle secretion was observed in *Lyngbya* sp. strain CFF-8, *Leptolyngbya* sp. strain CFF-9, *Leptolyngbya* sp. strain CFF-18 and *Leptolyngbya* sp. strain CFF-19.

Neutral lipids within cyanobacterial cells appeared red after staining with Nile red dye, while lipids were stained as green in color with BODIPY dye[40, 56]. *Oscillatoria* sp. strain CFF-6 showed significant detection of lipids among all isolated strains with a confocal laser scanning microscope[57]. *Leptolyngbya* sp. strain CFF-17 also showed a higher amount of lipids among all isolated *Leptolyngbya* strains. *Microcoleus* sp. strain CFF-16 showed a lesser amount of lipids, so detection was less with Nile red and BODIPY dyes. The same pattern of staining was observed when strains were dyed with BODIPY dye. Overall BODIPY dye showed a higher activity of lipid staining as compared to Nile red dye[58, 59].

Unsaturated lipids are of great concern because of their major role in the formation of biodiesel[60]. A crucial factor in affecting the kinematic viscosity of biodiesel is the saturation of fatty acids, which rises with increasing chain length and saturation. When exposed to oxygen in the air, double bonds found in the chains of fatty acids react. The degree of unsaturation, as well as the position and quantity of double bonds, significantly influence the fluidity of biodiesel. The amount and type of double bonds (*cis* or *trans*) as well as their position have an impact on the viscosity of unsaturated fatty acids. The fuel injection characteristics of biodiesel are greatly influenced by density. The EN 14214 standards set a density restriction of 0.860-0.900 g/cm^3 at 15℃, however, the ASTM D6751 standard makes no

Table 6 Biodiesel production and representative features of filamentous cyanobacterial isolates.

Strains	Weight of biomass (g)	Amount of diesel (mL)	Diesel/ Biomass%	Color	Viscosity	Odor
Oscillatoria sp. strain CFF-1	6.3 ± 0.9 (de)	1.2 ± 0.2 (g)	19.0 (gh)	Yellow	×	✓
Phormidium sp. strain CFF-2	8.0 ± 1.4 (f)	0.2 ± 0.1 (a)	2.0 (ab)	Yellow	×	×
Oscillatoria sp. strain CFF-3	2.5 ± 0.1 (abc)	0.4 ± 0.1 (bcd)	16.0 (cdefg)	Yellow	✓	×
Oscillatoria sp. strain CFF-4	3.2 ± 0.8 (bc)	0.6 ± 0.1 (de)	18.0 (efgh)	Yellow	✓	✓
Phormidium sp. strain CFF-5	6.9 ± 1.3 (ef)	0.2 ± 0.1 (a)	2.3 (ab)	Light yellow	✓	×
Oscillatoria sp. strain CFF-6	5.7 ± 0.9 (de)	2 ± 0.2 (h)	35.6 (i)	Yellow	✓	✓
Oscillatoria sp. strain CFF-7	3.7 ± 0.3 (c)	0.8 ± 0.2 (f)	21.8 (h)	Yellow	×	✓
Lyngbya sp. strain CFF-8	3.3 ± 0.3 (bc)	0.2 ± 0.1 (a)	6.2 (b)	Bluish green	✓	×
Leptolyngbya sp. strain CFF-9	3.9 ± 0.2 (c)	0.5 ± 0.1 (cd)	12.6 (c)	Light yellow	✓	×
Oscillatoria sp. strain CFF-10	2.6 ± 0.4 (abc)	0.5 ± 0.1 (cd)	18.6 (fgh)	Bluish green	×	✓
Oscillatoria sp. strain CFF-11	3.1 ± 0.2 (bc)	0.6 ± 0.1 (de)	18.1 (fgh)	Yellow	×	✓
Leptolyngbya sp. strain CFF-12	3.6 ± 0.1 (c)	0.6 ± 0.1 (de)	15.4 (cdefg)	Yellow	✓	×
Leptolyngbya sp. strain CFF-13	2.4 ± 0.7 (abc)	0.4 ± 0.1 (abc)	13.1 (cd)	Light yellow	✓	✓
Leptolyngbya sp. strain CFF-14	6.0 ± 0.1 (de)	0.8 ± 0.1 (f)	13.4 (cde)	Light yellow	×	×
Leptolyngbya sp. strain CFF-15	5.1 ± 0.1 (d)	0.8 ± 0.1 (ef)	14.2 (cdef)	Yellow	×	✓
Microcoleus sp. strain CFF-16	9.8 ± 1.1 (g)	0.2 ± 0.1 (a)	1.6 (a)	Light yellow	✓	×
Leptolyngbya sp. strain CFF-17	1.6 ± 0.2 (a)	0.3 ± 0.1 (ab)	17.3 (defgh)	Yellow	✓	✓
Leptolyngbya sp. strain CFF-18	1.6 ± 0.2 (a)	0.3 ± 0.1 (ab)	17.1 (cdefg)	Yellow	✓	✓
Leptolyngbya sp. strain CFF-19	2.2 ± 0.7 (ab)	0.3 ± 0.1 (ab)	12.9 (cd)	Yellow	✓	✓

Mean of 3 values ± Standard deviation, ✓ = presence, × = absence. In each column, figures followed by different letter(s) are significantly different by Duncan's multiple range test (DMRT) ($p < 0.05$).

mention of density. To manage fuel qualities, it is necessary to adjust the ratio of saturated to unsaturated fatty acids[61]. More than 40% methyl palmitoleate was observed in *Leptolyngbya* spp. strain CFF-12 and CFF-18[59, 62]. The process of harmonization does not appear to be challenging. Many countries that have established BDF (Biodiesel fuel) standards have adopted the EU EN14214 standard as their basis, which also serves as the foundation for the EAS–ERIA standard. As a result, the BDF standards frameworks in most countries are notably similar. FTIR technique was used for the determination of carboxylic acid functional group in the respective biodiesel obtained from the isolated strains. Detection of ester group (in the region of 1600-1740 cm^{-1}) is the fundamental unit of fatty acids[60]. Biodiesel yield obtained from consortia was significantly higher as compared to their individual strains (t test; $p = 0.02$). The strains in consortia provide symbiotic environment to each other as well as they have a greater ability of quorum sensing which enhance their biomass and lipid production. The highly promising results were obtained from the strains grown in field as compared to grown in lab (t test; $p = 3.48 \times 10^{-8}$). In the field, cyanobacteria face the natural environment while in lab there are proper sterile conditions. These organisms grow well in the natural open environment[62]. The selected isolated strains grown in different sodium nitrate conditions gave the better results with $BG11_{1.5\%NaNO3}$ media (ANOVA; $p = 0.009$) because this provides specific nutritional requirement of nitrate. At lower concentration of nitrate, strains are unable to grow properly while higher levels of nitrate are also lethal for the growth and production of lipids. The researchers performed a comparative study of *Phormidium* sp. FW01, *Phormidium* sp. FW02, *Oscillatoria* sp. FW01, and *Oscillatoria* sp. FW02 was conducted for lipid yield and biodiesel potential and reported 6.7, 8.2, 10.2, and 9.4% lipids, respectively. Under optimum abiotic conditions with 57% FAME output and desired fatty acids *Oscillatoria* sp. FW01 has been reported as promising feedstock for large-scale biodiesel synthesis. Natural fossil fuels sources are diminishing from planet earth because of frequent use in our daily lives. To overcome this disastrous change, there is a shift towards third generation biofuels from microalgae. Filamentous cyanobacteria play a better role in the production of lipids and biodiesel to compensate for the frequent use of limited fuels from natural fossils fuel for transport purpose. Significant challenges include finding acceptable

Table 7 Biodiesel production by selected cyanobacteria isolates cultivated in BG11 media containing different sodium nitrate concentrations.

Sr. No	Strain	Characters	0%	0.5%	1%	1.5%	2%
1.	*Oscillatoria* sp. strain CFF-1	Biomass (g)	12.2 ± 1.2 (c)	2.2 ± 0.9 (a)	5.5 ± 1.2 (b)	3.4 ± 1.1 (a)	7.2 ± 1.5 (b)
		Biodiesel (mL)	0.5 ± 0.1 (a)	0.4 ± 0.2 (a)	0.6 ± 0.2 (a)	1.4 ± 0.5 (b)	0.6 ± 0.2 (a)
		% Yield	4.1(a)	18.5(c)	10.9(b)	40.9(d)	8.3(ab)
2.	*Oscillatoria* sp. strain CFF-3	Biomass (g)	7.4 ± 1.2 (a)	8.3 ± 1.0 (a)	13.3 ± 1.3 (b)	8.5 ± 1.2 (a)	14.1 ± 1.7 (b)
		Biodiesel (mL)	0.8 ± 0.1 (ab)	0.5 ± 0.2 (a)	1 ± 0.23 (b)	3 ± 0.4 (c)	0.5 ± 0.2 (a)
		% Yield	10.8 (b)	6.1(ab)	7.5(ab)	35.3(c)	3.6(a)
3.	*Oscillatoria* sp. strain CFF-4	Biomass (g)	20.8 ± 1.6 (b)	16.2 ± 1.3 (ab)	21.1 ± 1.7 (b)	14.3 ± 1.1 (a)	17.4 ± 2.2 (ab)
		Biodiesel (mL)	2.6 ± 0.2 (b)	3.8 ± 0.3 (c)	4 ± 0.5 (c)	5.2 ± 0.8 (d)	1.6 ± 0.3 (a)
		% Yield	12.5(a)	23.5 (b)	18.9(b)	36.5(c)	9.2(a)
4.	*Oscillatoria* sp. strain CFF-6	Biomass (g)	7.4 ± 1.2 (a)	10 ± 1.2 (ab)	13.3 ± 1.3 (c)	11.1 ± 1.1 (bc)	14.1 ± 1.7 (c)
		Biodiesel (mL)	1.6 ± 0.2 (a)	3.8 ± 0.3 (b)	4 ± 0.5 (b)	5.2 ± 0.8 (c)	1.6 ± 0.3 (a)
		% Yield	21.6(b)	38 (c)	30.1(bc)	47.2(d)	11.3 (a)
5.	*Oscillatoria* sp. strain CFF-7	Biomass (g)	14.1 ± 2.0 (b)	13.9 ± 1.7 (b)	14.4 ± 2.2 (b)	3.7 ± 1.3 (a)	4.8 ± 2.9 (a)
		Biodiesel (mL)	0.7 ± 0.2 (a)	1 ± 0.4 (ab)	1.3 ± 0.6 (bc)	1.5 ± 1.0 (c)	1 ± 0.4 (ab)
		% Yield	5.0(a)	7.2(a)	9.1(a)	41.3(c)	20.8(b)
6.	*Oscillatoria* sp. strain CFF-10	Biomass (g)	14.0 ± 2.0 (b)	13.9 ± 1.7 (b)	14.4 ± 2.2 (b)	3.7 ± 1.3 (a)	5.2 ± 2.9 (a)
		Biodiesel (mL)	0.5 ± 0.1 (a)	0.4 ± 0.2 (a)	0.6 ± 0.2 (a)	1.4 ± 0.5 (b)	0.6 ± 0.2 (a)
		% Yield	3.6 (a)	2.9 (a)	4.2 (a)	38.6 (c)	12.5 (b)
7.	*Oscillatoria* sp. strain CFF-11	Biomass (g)	12.9 ± 1.9 (d)	9.8 ± 1.7 (c)	7.4 ± 1.9 (bc)	5.5 ± 0.9 (ab)	3.9 ± 1.2 (a)
		Biodiesel (mL)	0.8 ± 0.1 (ab)	0.5 ± 0.1 (a)	1 ± 0.3 (b)	2.1 ± 0.4 (c)	0.5 ± 0.2 (a)
		% Yield	6.2 (a)	5.2 (a)	13.5 (b)	38.2 (c)	13.1 (b)
8.	*Leptolyngbya* sp. strain CFF-12	Biomass (g)	20.8 ± 1.6 (b)	16.2 ± 1.3 (b)	20 ± 1.7 (b)	10 ± 1.0 (a)	17.5 ± 2.2 (b)
		Biodiesel (mL)	0.8 ± 0.1 (ab)	0.5 ± 0.1 (a)	1.2 ± 0.2 (b)	3 ± 0.4 (c)	0.5 ± 0.2 (a)
		% Yield	3.8(a)	3.1(a)	6 (a)	30 (b)	2.9(a)
9.	*Leptolyngbya* sp. strain CFF-17	Biomass (g)	12.2 ± 1.1 (d)	2.2 ± 0.9 (a)	5.6 ± 1.2 (bc)	3.4 ± 0.7 (ab)	7.2 ± 1.5 (c)
		Biodiesel (mL)	0.8 ± 0.1 (bc)	0.5 ± 0.2 (ab)	0.9 ± 0.2 (c)	1.2 ± 0.4 (d)	0.5 ± 0.2 (a)
		% Yield	6.6(a)	23.2 (b)	16.3 (b)	34.9 (c)	6.9 (a)
10.	*Leptolyngbya* sp. strain CFF-18	Biomass (g)	7.4 ± 1.2 (a)	8.3 ± 1.9 (ab)	13.3 ± 1.3 (c)	11.1 ± 1.8 (bc)	14.1 ± 1.7 (c)
		Biodiesel (mL)	0.8 ± 0.1 (a)	1.6 ± 0.3 (b)	3.8 ± 0.3 (c)	3.4 ± 0.6 (c)	1.6 ± 0.2 (ab)
		% Yield	10.9(a)	19.4(b)	28.6 (c)	30.8 (c)	11.4 (a)

Mean of 3 values ± Standard deviation. In each row, figures followed by different letter(s) are significantly different by Duncan's multiple range test (DMRT) ($p < 0.05$).

microalgae strains, the need for input resources, collecting biomass, the seasonal unpredictability of feedstock supplies, and choosing the right conversion technology. Due to environmental factors, lower intensity of sunlight results in less yield of cyanobacteria cells while high moisture content acts as a major challenge for energy-intensive drying stages[63–65]. Third-generation biofuel production from cyanobacteria is expensive due to increased interest from various private businesses, academic institutions, and government entities. Collaborative research projects have been focused on biodiesel production. Socioeconomic challenges, such as a lack of supportive government policies and efficient finance methods, are the main obstacles to progress in the biofuel industry[66].

Conflict of Interest

The authors declare there are no conflicts of interest.

Acknowledgment

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Table 8 Biodiesel production of selected filamentous cyanobacteria isolates grown in natural environment.

Strains	Wet biomass weight (g)	Amount of diesel (mL)	Diesel/Biomass (%)
Oscillatoria sp. strain CFF-1	21.5 ± 2.8 (b)	10 ± 2.8 (e)	46.5 (cd)
Oscillatoria sp. strain CFF-3	35 ± 2.5 (c)	13 ± 2.2 (a)	37.2 (a)
Oscillatoria sp. strain CFF-4	25 ± 1.5 (a)	11 ± 1.9 (bc)	44.0 (bc)
Oscillatoria sp. strain CFF-6	26 ± 2.5 (a)	12.5 ± 1.7 (c)	48.1 (cd)
Oscillatoria sp. strain CFF-7	17 ± 2.1 (bc)	8 ± 1.5 (a)	47.1 (a)
Oscillatoria sp. strain CFF-10	26 ± 2.8 (b)	12 ± 1.9 (f)	46.2 (e)
Oscillatoria sp. strain CFF-11	20 ± 3 (a)	9 ± 2.9 (d)	45.0 (d)
Leptolyngbya sp. strain CFF-12	34 ± 2.5 (a)	12 ± 1.2 (ab)	35.3 (a)
Leptolyngbya sp. strain CFF-17	21 ± 2.3 (a)	9 ± 1.5 (c)	42.9 (b)
Leptolyngbya sp. strain CFF-18	35 ± 2.5 (a)	14 ± 2.2 (c)	40.0 (cd)

Mean of 3 values ± Standard deviation. In each column, figures followed by different letter(s) are significantly different by Duncan's multiple range test (DMRT) ($p < 0.05$).

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Authors Contribution

Sara Janiad conducted the research work in the lab, wrote the research article. Mehboob Ahmed supervised and designed the research project, helped to draft the initial draft of the research article. Peter Lindblad helped in accomplishing the part of research work performed in the Uppsala University, Sweden.

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