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Enhancement of biodiesel potential in cyanobacteria: using agro-industrial wastes for fuel production, properties and acetyl CoA carboxylase D (*accD*) gene expression of *Synechocystis* sp.NN

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ABSTRACT

In this study, two freshwater cyanobacteria, *Oscillatoria* sp. 50A and *Synechocystis* sp. NN have been evaluated for biodiesel production. Among the two cyanobacteria, *Synechocystis* sp. NN was isolated, identified by its 16S rRNA gene sequencing. Effects of sodium bicarbonate (SBC), tannery effluent (TE), coir pith (CP) and light stress (L1) on biomass and lipid production of *Synechocystis* sp. NN were studied. Result showed that maximum biomass productivity of 18.7 ± 0.9 mg/L/day (1.9 folds) was observed in TE supplemented BG-11 media than normal BG-11 media. Meantime, maximum lipid productivity of 2.6 ± 0.4 mg/L/day (1.4 folds) was observed in CP supplemented BG-11 media than normal media. Further, fatty acid composition analyses by GC–MS showed that C16, C18:1 in *Oscillatoria* sp. 50A and C16, C20:1, C22:1 in *Synechocystis* sp. NN were predominant and the fuel properties were also in accordance with the international standards. Besides gene expression of acetyl CoA carboxylase D of *Synechocystis* sp. NN, analyzed by RT-PCR revealed that transcripts of *accD* were up-regulated by 1.2–4.7 folds in different media conditions. The findings of this study showed that *Synechocystis* sp. NN can be utilized as a suitable feedstock that is amenable for cultivation using wastes as nutrient source.

1. Introduction

Demand for alternative fuel is getting great deal of significance worldwide, owing to the excessive consumption of fossils (non-renewable resources) and global warming. Prolonged use of fossil fuels has resulted in increased green house gas emissions that have eventually led to climate change globally [1]. In the recent years, prices of petroleum have escalated due to their huge energy demand from developing countries, diminishing fossil reserves and geo-political instability in oil exporting countries [2,3]. Thus, exploring renewable feedstock for fuel production is a major task for researchers. Fuel from algae and cyanobacteria is one such important renewable biological source, which is being used in developed countries. In this context, cyanobacteria are perhaps the most attractive prokaryotic bacteria, as they utilize cheap nutrients like industrial wastes. Similarly, algae show higher growth and lipid production in wastewater, which translates to better environmental management as improper disposal of industrial waste such as tannery effluent (TE), which is rich in inorganic chloride, sulphide, ammonia, fats, surfactants, arsenic and chromium, poisons freshwater sources and affects terrestrial and aquatic life [4–6]. Currently, researches focusing on wastewater utilization for the development of higher algal biomass that can be used as a renewable feedstock for biofuel production, are gaining much importance.

Diesel and gasoline can be comfortably replaced with biodiesel and bio-ethanol respectively in vehicles, with fewer or no modification. They offer the dual benefit of maintaining engine performance while being produced from renewable feedstocks [7]. Biodiesel production involves a chemical reaction called transesterification in which triglycerides react with methanol or ethanol in the presence of a catalyst to yield fatty acid methyl esters (FAME) [8]. As large agricultural lands will be required for the cultivation of plant feedstocks, and since there is always the possibility of

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competition with food crops, production of biodiesel from plant sources has been getting lesser importance in recent decades [9]. Consequently, cyanobacteria and microalgae are being considered as a raw material for biodiesel production owing their copious benefits including CO_2 mitigation, land availability and high oil yield [10,11]. In contrast with microalgae, cyanobacterial lipids can be enhanced by over-expressing proteins in thylakoid membranes which in turn improve the biomass and photosynthetic rates in the log phase [12]. Biodiesel fuel properties greatly depend on the ester moiety, obtained from alcohol during the transesterification reaction and the fatty acid profile [13]. Thus, biodiesel produced from a green renewable feedstock would be the best solution for mitigating the mounting fuel demand and global warming crisis. This study was designed to characterize the fatty acid profile of two freshwater cyanobacteria and to evaluate the biodiesel fuel properties based on their fatty acid composition. Attempts were also made to enhance the biomass and lipid productivity in unicellular cyanobacteria *Synechocystis* sp. NN by supplementing the growth media with sodium bicarbonate (SBC), tannery effluent (TE), coir pith (CP), and light stress (L1). Furthermore, the *accD* gene expression was also analyzed under the conditions of light stress and waste supplementation using semi-quantitative RT-PCR.

2. Materials and methods

2.1. Cyanobacterial isolation and biomass cultivation

Cyanobacteria samples were collected from fresh water bodies in the vicinity of Madurai, Tamil Nadu, India and were enriched in BG-11 media [14]. Samples were serially diluted and streaked onto BG-11 agar plates and incubated for 15–20 days at 25 °C under constant illumination of alternate photoperiod (12 h light:12 h dark) at 1500 lux. Cultures were monitored microscopically and an axenic colony was isolated by repeated streaking. Axenic cultures were then transferred to 5 mL of BG-11 liquid medium and sub cultured to 100 mL BG-11 medium. An oleaginous unicellular cyanobacetrial isolate NN and a filamentous cyanobacterium *Oscillatoria* sp. 50A were subjected for further evaluation of biodiesel production in this study.

2.2. High resolution – scanning electron microscopy (HR–SEM)

In order to visualize the surface topology, isolate NN was imaged under HR-SEM (Carl Zeiss EVO 18, Germany). Cells were suspended in carbon tape and air dried. Filament was adjusted for optimum resolution in conducting mode under the magnification of 10,000X and the image was captured using Backscattered Electron Detector.

2.3. Genomic DNA extraction and molecular characterization

Genomic DNA was extracted from the log phase cultures of isolate NN using modified method [15]. 16S rRNA gene amplification was performed using genomic DNA (50 ng) as template and universal primers 27F and 1492R. Briefly, 50 μl reaction comprising 25 μl of ReadyMix™ Taq PCR Reaction Mix (Sigma–Aldrich, St. Louis, MO), 17 μl of molecular biology grade water (Hi–media, Mumbai, India), 2 μl of forward and reverse primers each (0.4 μM) and 4 μl of genomic DNA was used. 16S rRNA gene specific forward 5′ – AGAGTTTGATCCTGGCTCAG – 3′ and reverse primers 5′ – CGTTACCTTGTTACGACTT – 3′ were used. PCR was carried out in Nexus Gradient Mastercycler (Eppendorf, Germany) with the following PCR program: initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. A negative control was also run along with PCR using sterile water as template. PCR products were separated by gel electrophoresis on 1.2% agarose gel for 30 min at 50 V in an electrophoretic apparatus. Amplicon with expected size was visualized under UV transilluminator by ethidium bromide staining and purified using GenElute kit (Sigma–Aldrich, St. Louis, USA). PCR amplicons were sequenced using Sanger's dideoxy sequencing method and subjected to BLAST (www.ncbi.nlm.nih.gov) to identify the homologous sequence by comparing the query coverage and e-value. The identified 16S rRNA gene sequence of isolate NN was submitted to GenBank (http://www.ncbi.nlm.nih.gov/).

2.4. Study of biomass and lipid enhancement in Synechocystis sp. NN

In order to enhance the biomass and lipid production in *Synechocystis* sp. NN, cells were grown in BG-11 medium was supplemented with sodium bicarbonate (10 mM, 20 mM and 30 mM), tannery effluent (TE; 1.3 mL/L, 2.7 mL/L and 4 mL/L), coir pith (0.6 g/L) and also subjected to light stress (2000 lux). TE was collected from Subramaniam & Co Leather Industry Pvt. Ltd, Dindigul, Tamil Nadu, India. An initial inoculum of 12 mg (dry cell weight) of *Synechocystis* sp. NN was equally added to the flasks containing respective supplements and maintained as foresaid culture conditions for 40 days. To identify the functional groups present in the TE, FT-IR analysis was performed and the key physico–chemical parameters of TE like pH, total dissolved solids and alkalinity were evaluated.

2.5. RNA extraction and cDNA synthesis

Frozen *Synechocystis* sp. NN cells were ground using mortar and pestle with liquid nitrogen and the cell lysate was used to extract the total RNA (RNeasy® Mini Kit, QIAGEN) by following the manufacturer's protocol. The extracted RNA was quantified using Biophotometer (Eppendorf, Germany) and its purity was verified by measuring absorbance at 260/280. cDNA synthesis was performed using 4.5 μg of DNAase treated total RNA using random hexamers of RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) by following the manufacturer's protocol.

2.6. Semi-quantitative reverse transcriptase – PCR (RT-PCR)

In order to study the gene expression of *Synechocystis* sp. NN under control BG-11 medium and lipid productivity enhanced media conditions, semi-quantitative RT-PCR was performed. 16S rRNA gene was used as an internal control. Primers used in this study were provided in Table 1. Briefly, a total of 25 μl reaction was performed comprising 12.5 μl AMPLIQON Master Mix, 8.5 μl of Nuclease Free Water, 1 μl of forward and reverse primers each (0.4 μM) and 2 μl of cDNA. PCR was carried out during exponential phase [initial denaturation of 94 °C for 4 min followed by 38 cycles of denaturation at 94 °C for 45 s (12 cycles for internal control gene) and annealing at 60 °C for 45 s]. PCR products were electrophoresed and visualized under gel documentation unit. Densitometry software ImageJ was used to analyze the difference in band intensities of *accD* and 16S rRNA genes.

Table 1
Primer sets used for semi-quantitative RT-PCR.

Gene	Primer	Sequence	Product size
accD	Forward primer	GGTGGAGCCAGAATGCAGG	153bp
accD	Reverse primer	CAACATGGCAAAGCTGGCAGT	
16S rRNA	Forward primer	TAAAGCGTCCGTAGGTGGCATTAC	143bp
16S rRNA	Reverse primer	TTCCCGATATCTACGCATTTCACC	

2.7. Growth and lipid quantification

Growth parameters like biomass productivity (mg/L/day), lipid productivity (mg/L/day) and lipid content (%) were calculated for the cells harvested in the exponential phase. Known volume of oven dried cells of filamentous isolate 50A was used for lipid quantification. Unicellular isolate NN was passed through pre-weighed GF/A glass filter (Whatman™ UK) followed by overnight drying at 50 °C and difference of dried filter paper was measured. Biomass productivity (1) was calculated by measuring initial and final dry weight of filter paper whereas lipid productivity (2) was calculated from initial and final weight of dry lipids extracted [16]. Total lipid content was expressed as percentage of lipids per known volume of biomass.

$$\text{Biomass productivity (BP)} = [(BP_2 - BP_1)/T_2 - T_1] \quad (1)$$

$$\text{Lipid productivity (LP)} = \text{BP} \times \text{Lipid content in \%} \quad (2)$$

Where BP_2 is the final weight of dry filter, BP_1 is the initial weight of filter, T_2 and T_1 are final and initial days of cell harvesting respectively.

2.8. Lipid extraction and FAME preparation

Cells were subjected to lipid extraction [17] using 5 mL of chloroform: methanol (2:1, v/v) by grinding the cells in mortar and pestle. Extracts were centrifuged repeatedly at 8000 rpm for 15 min until the cell pellet became colorless. 1% NaCl was added to the extract to allow phase separation which was carried out in a separating funnel under vigorous shaking. The lower organic phase containing lipid was separated, evaporated and quantified gravimetrically. All the experiments were performed in duplicates. FAME was prepared by transesterification of known volume of lipids [2]. Briefly, lipids were boiled for 30 min with 1 mL of saponification reagent containing 15 g NaOH in 100 mL of methanol: water (1:1, v/v). Following which they were boiled with 2 mL of methylation reagent comprising methanol: 6 N HCl (1:1.18, v/v) at 80 °C for 20 min. Contents were cooled and FAME was extracted with 1 mL of hexane. Finally, the extracted FAME was washed with 3 mL of wash solution (1.2% NaOH, w/v). In order to compare the fatty acid profiles, *Jatropha* sp. a commercial biodiesel feedstock was also used along with the isolates (*Oscillatoria* sp. 50A and *Synechocystis* sp. NN in this study). *Jatropha* oil (10 mg) was transesterified and its FAME profile was also characterized.

2.9. Gas chromatographic – mass spectrometry (GC–MS) analysis

GC–MS analysis was performed at Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology Madras (IITM), Chennai, India (www.saif.iitm.ac.in). FAME (1 μl) was injected in gas chromatograph (Agilent Technologies 6890, N Series, Santa Clara, CA) with electron ionization detector (EI–MS, JEOL GC MATE-II, JEOL, Ltd., Tokyo, Japan) equipped with HP-5 MS column, quadruple double-focusing mass analyzer and photon multiplier tube detector. The *m/z* ratio of the FAME peaks of individual strains and the standard Supelco 37 component FAME mix (Sigma–Aldrich, St. Louis, USA) were compared against the National Institute for Standards and Technology (NIST) database. Area normalization method was employed to quantify the relative percentage of individual fatty acids and all the samples were analyzed in duplicates.

2.10. Evaluation of biodiesel fuel properties

Important fuel properties were evaluated for both the strains used in this study from their fatty acid profile using empirical equations previously reported [18,26]. The properties include saponification value (SV), iodine value (IV), cetane number (CN), degree of unsaturation (DU), long chain saturation factor (LCSF), cold filter plugging point (CFPP), cloud point (CP) and pour point (PP).

3. Results and discussion

3.1. Isolation, purification and molecular characterization of cyanobacteria

Freshwater indigenous cyanobacterium was isolated, identified and evaluated for its biodiesel production based on its fatty acid profile. The unicellular isolate NN was found to be spherical in shape comprising both dividing and non-dividing cells. HR-SEM image revealed the size of the spherical cell to be about 2 μm and smooth cell wall that resembles unicellular cyanobacterium *Synechocystis* sp (Supplementary Fig. 1). In addition to the morphological identification, molecular identification by 16S rRNA gene sequencing and BLAST analysis of isolate NN was performed to determine the isolate. The BLAST results showed that the unicellular isolate NN was 91% homologous to *Synechocystis aquatilis* 1LT32S04 (Acc No: FM177503.1) with 100% query coverage, which was then submitted to GenBank database with the accession number KM061377.1.

3.2. Effects of SBC, TE, CP and L1 in biomass and lipid production of Synechocystis sp. NN

Large scale algal biomass and biodiesel production involves several criteria, among which biomass and lipid productions are crucial. In this perspective, *Synechocystis* sp. NN with notable higher lipid content (18.9 ± 2.8%) than *Oscillatoria* sp. 50A (9.6 ± 0.9%) was evaluated further for enhanced biomass and lipid under supplementation with SBC, TE, CP and L1 conditions. Physico-chemical parameters of TE were found to be TDS (1.2 g/L), pH (8) and alkalinity (1325 mg/L of $CaCO_3$). Moreover, FTIR analysis of TE revealed that it contains Ar–OH, carboxylic acid and amide stretches. Experimental results showed that BG-11 media supplemented with 4 mL/L of TE resulted in the maximum biomass productivity of 18.7 ± 0.9 mg/L/day which is 1.9 folds more than control BG-11 media (10 ± 0.3 mg/L/day) as shown in Fig. 1. Moreover, 1.3 mL/L and 2 mL/L TE also resulted in higher biomass productivities of 17.6 ± 0.4 and 14.7 ± 0.9 mg/L/day respectively (1.75 and 1.5 folds respectively) than control. In case of lipid productivity, supplementation of BG-11 media with coir pith resulted in maximum lipid productivity of 2.6 ± 0.4 mg/L/day which is 1.4 folds higher than the control condition (1.9 ± 0.2 mg/L/day) as shown in Fig. 2. In addition, 4 mL/L TE also resulted in higher lipid productivity of 2.5 ± 0.6 mg/L/day which is 1.3 folds higher than the control. Previously, *Spirulina* sp. was mass cultivated in TE waste stabilization pond resulting with the photosynthetic productivity of 9000 mgm^2day^{-1} carbon fixation [19]. As far as lipid content is concerned, no remarkable increase was observed in all the nutrient/waste supplementation and light stress conditions (Fig. 1). Furthermore, subjecting *Synechocystis* sp. NN to light stress condition resulted in a considerable increase in biomass productivity of 15.8 mg/L/day (1.6 folds higher than normal light condition). When *Synechocystis* sp. NN in BG-11 media was supplemented with sodium bicarbonate, biomass and lipid production decreased in all the concentrations. In contrast, microalga *Tetraselmis suecica* and

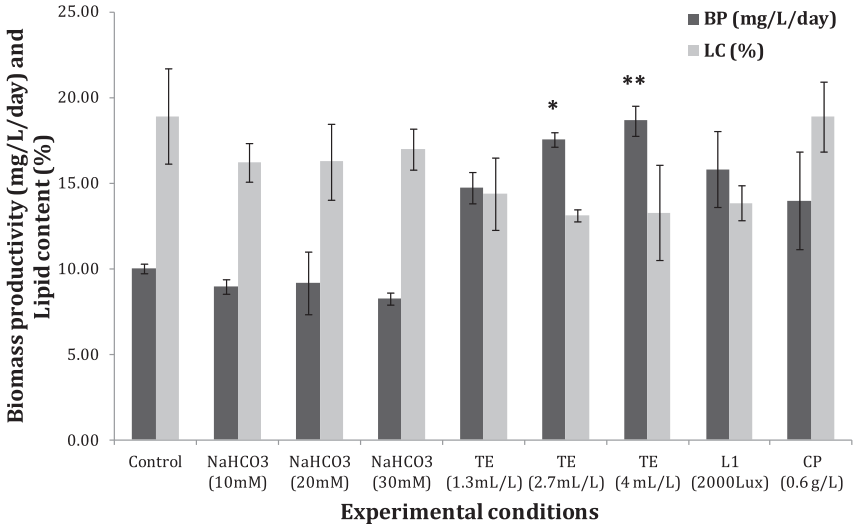

Fig. 1. Biomass productivity (mg/L/day) and lipid content (%) of *Synechocystis* sp. NN grown under different media conditions ($NaHCO_3$ – sodium bicarbonate, TE – tannery effluent, L1 – light stress, CP – coir pith). Data here represents the mean values of triplicates and bar shows standard error. Asterisk denotes the statistical significance $P < 0.05$ (*) and $P < 0.01$ (**).

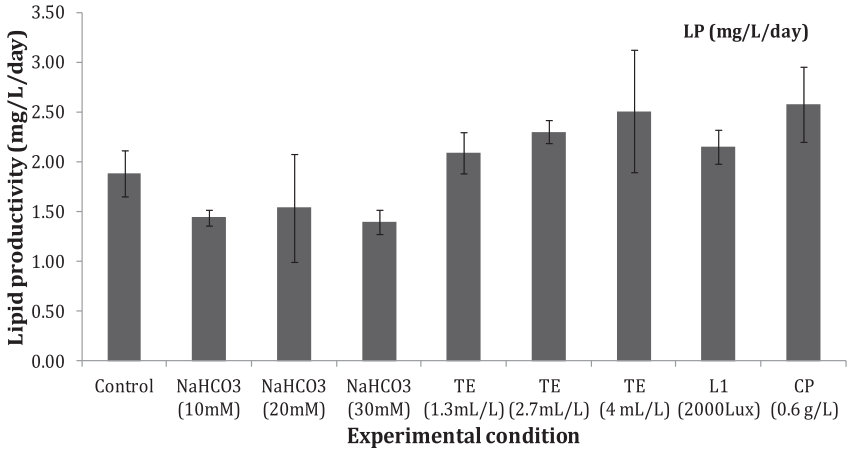

Fig. 2. Lipid productivity (mg/L/day) of *Synechocystis* sp. NN grown under different media conditions. ($NaHCO_3$ – sodium bicarbonate, TE – tannery effluent, L1 – light stress, CP – coir pith). Data represents the mean values of triplicates and bar represents standard error.

Nannochloropsis salina, when grown in f/2 medium supplemented with sodium bicarbonate exhibited higher final mean cell abundance, increased total cellular pigments and higher total cellular fatty acid contents [20]. In addition, *Nostoc calcicola* was found to have higher growth rates during the addition of 75 mM $NaHCO_3$ and also found that the strain can tolerate up to 250 mM $NaHCO_3$ although the growth rate declined at high $NaHCO_3$ concentrations [21].

3.3. Transcript analysis of accD in Synechocystis sp. NN under different media conditions

Acetyl-CoA carboxylase (ACCase) catalyzes the first rate-limiting step in the fatty acid biosynthesis leading to the formation of malonyl-CoA from acetyl-CoA. The malonyl- CoA conversion occurs in two stages, the first being the carboxylation of biotin which is catalyzed by biotin carboxyl carrier protein (*accB*) and biotin carboxylase activity (*accC*). The above reaction is followed by the second half-reaction: the carboxyl group transfer from carboxylated biotin to acetyl-CoA that forms malonyl-CoA. α-Carboxyl transferase (*accA*) and β-Carboxyl transferase (*accD*) catalyzes the second reaction [22]. The functional role of *accD* in lipid biosynthesis was revealed in earlier studies pertaining to over-expression of genes in microalgae and plants [23,24]. Semi-quantitative RT-PCR based *accD* gene expression analysis of *Synechocystis* sp. NN grown in control BG-11 media and different waste supplemented

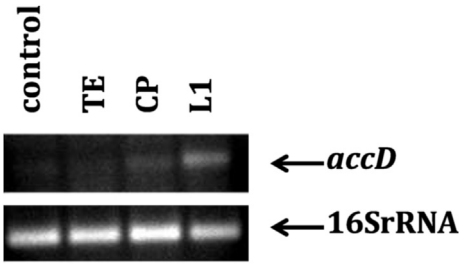

Fig. 3. Semi-quantitative RT-PCR analysis of *accD* gene of *Synechocystis* sp. NN under different media conditions (TE – 4 mL/L tannery effluent, CP – 0.6 g/L coir pith and L1 – light stress).

media conditions (TE, CP, L1) were shown in Fig. 3. Results showed that, light stress had resulted in significant increase in *accD* transcript level (4.7 ± 1.4 folds) while coir pith supplemented BG-11 media showed a noticeable increase in the transcript level of *accD* (2.3 ± 0.2 folds) than control that substantiates the increase in lipid production under treated conditions (Fig. 4). Similar increase in *accD* transcript level (38.9 folds) was observed in the microalgae, *Chlorella pyrenoidosa* in the lipid production stage during nitrogen starvation [25].

3.4. Fatty acid characterization by GC–MS analysis

The proportions of saturated and unsaturated fatty acid greatly influence the fuel properties of biodiesel [26]. The relative percentage of fatty acids present in *Jatropha* sp., *Oscillatoria* sp. 50A and *Synechocystis* sp. NN are shown in Fig. 5. Results showed that C16:0 (palmitic acid) and C18:1 (oleic acid) were found to be predominant fatty acids of *Oscillatoria* sp. 50A, whereas C16:0, C20:1 (Eicosenoic acid) and C22:1 (Erucic acid) were found to be dominant fatty acids in *Synechocystis* sp. NN, which makes them suitable feedstocks for high quality biodiesel. The fatty acid composition of the most common biodiesel feedstock *Jatropha* sp. consists of C18:2 (Linoleic acid) which is not suitable biodiesel for engine biodiesel. Further, total saturated fatty acid (SFA) of *Synechocystis* sp. NN was highest (38 ± 6.8%) than *Oscillatoria* sp. (26 ± 8.6%) and *Jatropha* sp. (10 ± 2.2%), proving that it favors the production of good quality biodiesel. Moreover, *Oscillatoria* sp. 50A and *Synechocystis* sp. NN contain undetectable levels of polyunsaturated fatty acid (PUFA) that makes them more suitable for blending with conventional petro-diesel. Similar results were observed in *Myxosarcina* sp. cyanobacteria with undetectable level of PUFA with higher proportions of monounsaturated fatty acid (MUFA) [26,27].

3.5. Biodiesel fuel properties

The biodiesel fuel properties are directly associated to the FAME profile. Key biodiesel fuel properties of *Synechocystis* sp. NN, *Oscillatoria* sp. 50A, *Jatropha* sp. identified by their fatty acid profile are shown in Table 2. Generally, high percentage of SFA and MUFA translates to better oxidative stability and energy yield from biodiesel. Although the presence of higher amount of PUFA provides good cold-flow properties, on the downside it results in poor oxidation stability [28]. Hence, degree of unsaturation (DU) is an indispensable property as it determines the oxidative stability of biodiesel. In this study, biodiesel fuel parameters namely, saponification value (SV), iodine value (IV), cetane number (CN), degree of unsaturation (DU), long chain saturation factor (LCSF), cold filter

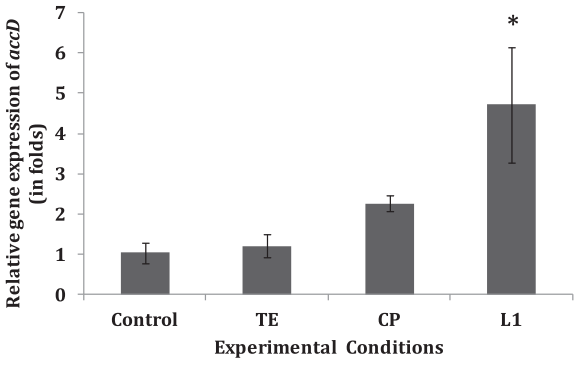

Fig. 4. Relative gene expression of *accD* gene of *Synechocystis* sp. NN grown in different media conditions (TE – 4 mL/L tannery effluent, CP – 0.6 g/L coir pith and L1 – light stress). $P < 0.05$ (*).

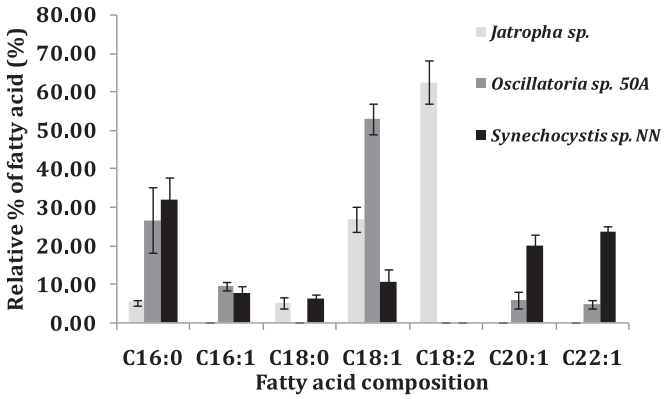

Fig. 5. Fatty acid composition of *Jatropha* sp., *Oscillatoria* sp. 50A and *Synechocystis* sp. NN analyzed by GC–MS. Data represents the mean values of duplicates and bar shows standard error.

plugging point (CFPP), cloud point (CP) and pour point (PP) were assessed from the FAME profile and were tabulated (Table 2). The SV of *Synechocystis* sp. NN and *Oscillatoria* sp. 50A were found to be 193.4 ± 0.6 and 202.8 ± 2.3 mg KOH g^{-1} of oil respectively, which is the amount of KOH (in milligrams) needed for the saponification of 1 g of oil. These values were found similar to the SV values reported earlier in cyanobacteria and microalgae [2,18]. The CN of biodiesel denotes the ignition quality; higher the saturated fatty acid content, higher the CN and consequently, better is the combustion quality of the biodiesel. The CN of *Synechocystis* sp. NN and *Oscillatoria* sp. 50A were found to be 58.5 ± 1.4 and 63.2 ± 1.4 respectively, which is higher compared to that of *Jatropha* sp. Higher the number of double bonds in the fatty acids, the higher the IV (equivalent to the no. of grams of iodine consumed by 100 g of oil), which determines the oxidative stability of the biodiesel. The IV of *Synechocystis* sp. NN and *Oscillatoria* sp. 50A were found to be 50.5 ± 6.4 and 65.6 ± 7.5 respectively which is much lower than IV of *Jatropha* sp. This indicates cyanobacetrial biodiesel's stability against oxidation. Further, cold flow properties like LCSF, CFPP, CP and PP of *Oscillatoria* sp. 50A and *Synechocystis* sp. NN which possess long chain saturated fatty acid, were found to be in accordance with biodiesel standards. Thus the fuel parameters of *Oscillatoria* sp. 50A and *Synechocystis* sp. NN biodiesel were found to match with the biodiesel fuel specifications given by the regulatory international standards, ASTM D6751 in the U.S. and EN 14214 in Europe. Based on this study, *Synechocystis* sp. NN yielded 18.9% lipid content with 38 ± 6% SFA and 62 ± 9.7 MUFA also possesing the capability to utilize agro-industrial wastes thus this study emphasizing *Synechocystis* sp. NN as a feasible, alternate, renewable and eco-friendly biodiesel feedstock. Lipid contents of several cyanobacterium reported were *Aphanothece microscopic* Nägeli – 8%, *Spirulina maxima* LB 2342–4.1%, *Synechococcus* sp. PCC7942–26.9% and *Trichormus* sp. CENA77–23.7% [29] that are closer to the yield with *Synechocystis* sp. NN used in the current study. Fatty acid compositions of both the cyanobacterial strains evaluated in this study were found to have no/undetectable level of PUFA that would result in high quality biodiesel production which would make the blending process easier. In addition, a versatile multi-dimensional strategy can be employed in large scale production of cyanobacteria for environmental CO_2 capturing by utilizing industrial wastes towards production of sustainable biofuels and high value bio-products.

4. Conclusion

Based on the above said observations, *Synechocystis* sp. NN can

Table 2
Biodiesel fuel properties of *Jatropha* sp., *Oscillatoria* sp. 50A and *Synechocystis* sp. NN assessed by fatty acid analysis by GC–MS.

Biodiesel fuel parameter	*Jatropha* sp.	*Oscillatoria* sp.	*Synechocystis* sp.
Saponification value (mg KOH g^{-1} of oil)	199.7 ± 0.07	202.8 ± 2.3	193.4 ± 0.6
Iodine value (g I_2 100 g^{-1} of oil)	137.6 ± 6.99	65.6 ± 7.5	50.5 ± 6.4
Cetane number	42.7 ± 1.6	58.5 ± 1.4	63.2 ± 1.4
Degree of unsaturation (wt%)	152. ± 7.7	73.3 ± 8.6	61.9 ± 6.8
Long chain saturation factor (°C)	3.2 ± 0.8	2.7 ± 0.9	6.2 ± 1.2
Cold filter plugging Point (°C)	−6.6 ± 2.6	−8.1 ± 2.7	3.1 ± 3.7
Cloud point (°C)	−2.2 ± 0.4	9.1 ± 4.5	11.9 ± 2.9
Pour point (°C)	−9.2 ± 0.4	3 ± 4.9	6.1 ± 3.2

serve as better feedstock for biodiesel production due to its higher biomass and lipid productivities by the waste utilization such as TE and CP as an added advantage. To the best of our knowledge, this is the first report stating the gene expression of *accD* of *Synechocystis* sp. NN under waste supplemented media conditions.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.renene.2016.02.038.

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