International Journal of Ecology and Environmental Sciences 47: 251-258, 2021
ISSN: 2320-5199 (Online)

Biodiesel Production of *Microseira woelli* Isolated from Fresh Water Pond

DHANALAKSHMI, J.* AND. P. JEEVAN

Department of Microbiology, J. J. College of Arts & Science (Autonomous), J. J. Nagar, Sivapuram, Pudukkottai, 622 422, Tamil Nadu, India.
E-mail: 2015ganesang@gmail.com, pjjevanmicro@gmail.com
Corresponding author

ABSTRACT

Algal biofuel or algal oil is an alternative to liquid fossil fuels that uses algae as the source of energy and they are considered as a replacement for biofuel sources such as corn and sugarcane. This study presents the isolation of algal strain from fresh water ponds in Pudukkottai District, Tamil Nadu, India. The algal strain was confirmed as *Microseira wollei* after PCR amplification and sequencing of the 16s rRNA gene of the DNA isolated from the species. The structure of the strain was observed using a trinocular microscope. Biodiesel was produced from *M. wollei* by lipid extraction and allowed for the process of trans-esterification. The biodiesel production of was *M. wollei* was confirmed by GC-MS analysis which showed high quantity of methyl ester compounds.

Keywords: Algae, *Microseira woelli*, biodiesel, 16s rRNA gene, trans-esterification, GC-MS

INTRODUCTION

The energy crisis and greenhouse gas emissions throughout the world have driven the search for alternative and eco-friendly renewable energy sources (Passoth et al. 2014). Biodiesel derived from biomass is an emerging source of fuel, and the global application of biodiesel in the transport sector was tremendously increasing (Valentine et al. 2012) and Kim et al. 2004). The Microalgae biofuel is identified as one of the renewable energy sources for sustainable development, having the potential to replace fossil fuels (Klutz et al. 2017) and Gnansounou 2010). Microalgae biofuel was devoid of the major drawbacks associated with oil crops and lignocelluloses-based biofuels. They are economically viable and cost-competitive which require minimal water use and mitigate atmospheric CO_2 (Glithero et al. 2015). However, biofuels are currently mainly produced from so-called first-generation substrates such as sugar cane, wheat grain, or vegetable oils (Sims et al. 2010) and (Townsend et al. 2017), i.e. resources that also can be used as human food. This use of food crops has been criticized due to potential food versus fuel competition (Jorgensen et al. 2018), (Panoutsou et al. 2017) and (Kasting. 2013). Therefore, substantial research has been conducted to establish biofuel production. Microalgae can be a rich source of carbon compounds, which can be utilized in biofuels, health supplements, pharmaceuticals, and cosmetics (Kadam et al. 2000). Microalgae produce a wide range of bioproducts, including polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants (Talebnia et al. 2010). Cyanobacteria possess certain properties which have entitled them to be one of the most promising feedstock for bioenergy generation (Tishler et al. 2015) i.e., They contain considerable amounts of lipids, which are mainly present in the thylakoid membranes, possess higher photosynthetic levels and growth rates compared to other algae and higher plants and cyanobacteria grows easily with basic nutritional requirements; they can survive if supplied with air [N_2 (nitrogen-fixing strains) and CO_2], water, and mineral salts (especially phosphorous-containing salts) with light as the only energy source.

The accumulation of lipids in algae occurs when the organism is under stress (e.g. nutrient deprivation) and in the stationary growth phase (Passoth et al. 2013). Another secondary advantage is that cyanobacteria, being prokaryotes, can much more readily be genetically engineered to enhance the production of biofuels as opposed to eukaryotic algae

(Nilsson 2000). Lipid production in thylakoid membranes is associated with high rates of rapid growth and photosynthesis, which is ideal for biodiesel production. It is produced by a trans-esterification reaction, in which triacyl glycerides react with short-chain alcohols to form alkyl esters (Falcon et al. 2010). Under normal conditions, cyanobacteria are generally cultured in vitro in a BG-11 medium at 30°C under agitation and illumination of 50 µmol photons m^{-2} s^{-1}, the culture being the main form of biomass obtainment (Fischer et al. 2016). The production of oils from microalgae and cyanobacteria biomass is related to the cultivation conditions to which they are subjected and can stimulate or suppress lipid productivity (Schirrmesiter et al. 2016). The optimization of components of crop media, such as CO_2, H_2O, N_2 and factors such as light intensity, pH and temperature influence the growth of biomass and the lipid content. Studies have been directed to cultivation conditions to increase lipid and biomass production and thus to adjust the profile of fatty acids, affording better-quality biodiesel (Mazard et al. 2016). The biosynthesis of fatty acid-based biofuels in cyanobacteria includes two steps, production, and trans-esterification of fatty acids (FAs) to form alkyl FA esters (Rittmann 2008). Considering that fuel properties are largely dependent on the FA composition of the feedstock from which biodiesel is prepared, the FA profile was employed as a screening tool for the selection of cyanobacterial lipids with high amounts of monounsaturated fatty acids (Murata et al. 1993, Bruno et al. 2012). This work presents the biodiesel production of algae isolated from fresh water ponds of Pudukkottai district, Tamil Nadu.

MATERIALS AND METHODS

Collection of samples

The fresh water alga was isolated from Sri Brahadhambal temple pond located at 10.3915^0 N, 78.8005^0 E in the district of Pudukkottai, Tamil Nadu, India. The various algal strains were collected from the fresh water ponds and samples were stored under sterile conditions. The fresh water was collected in distilled polyethylene bottles. The collected samples were shifted to the laboratory rapidly for analysis of various physicochemical parameters and heavy metals analysis as per the methods prescribed in APHA (American Public Health Association), 1912. The collected samples were presented for further analysis.

Figure 1. Mass culture of isolated algae

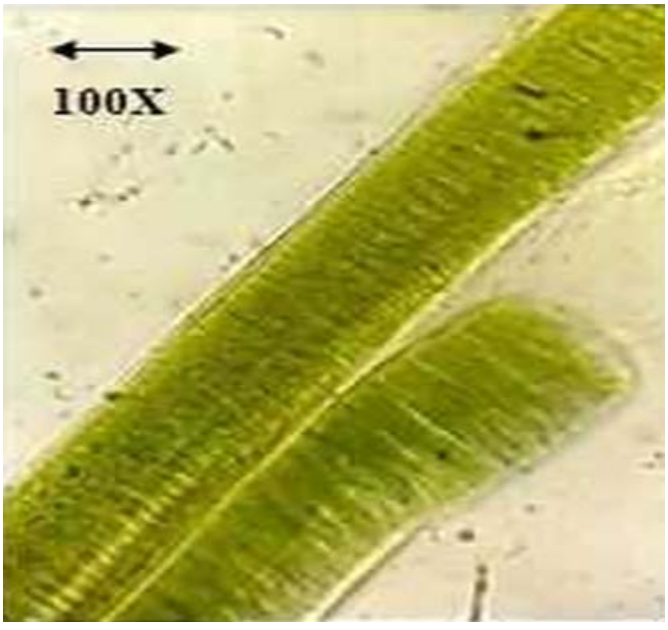

Figure 2. *Microseira woelli*

Isolation and Identification of fresh water algae

The isolated algal samples were made into a homogenous suspension in a sterile environment and were seeded on to the BG-11 medium. Algae appearing on the medium were wet mounted over a clean slide and observed under the microscope. Figure 1 shows the mass culture of isolated algae. Photomicrographs were also made. Figure 2 represents *Microseira woelli*. The algae were identified by using standard manual like the Manual of Freshwater Algae of Tamil Nadu (Perumal and Anand 2009).

Isolation of genomic DNA and determination of DNA quality

DNA was isolated from each separated algal culture using inorganic extraction by the standardized salting-out method using NaCl and combining Proteinase K, which yields more DNA. Quantity of the extracted DNA was checked in UV spectrophotometer (SHIMADZU, JAPAN) at an optical density (OD) 260 nm and 280 nm was analyzed. Figure 3 shows the Agarose gel electrophoresis of DNA.

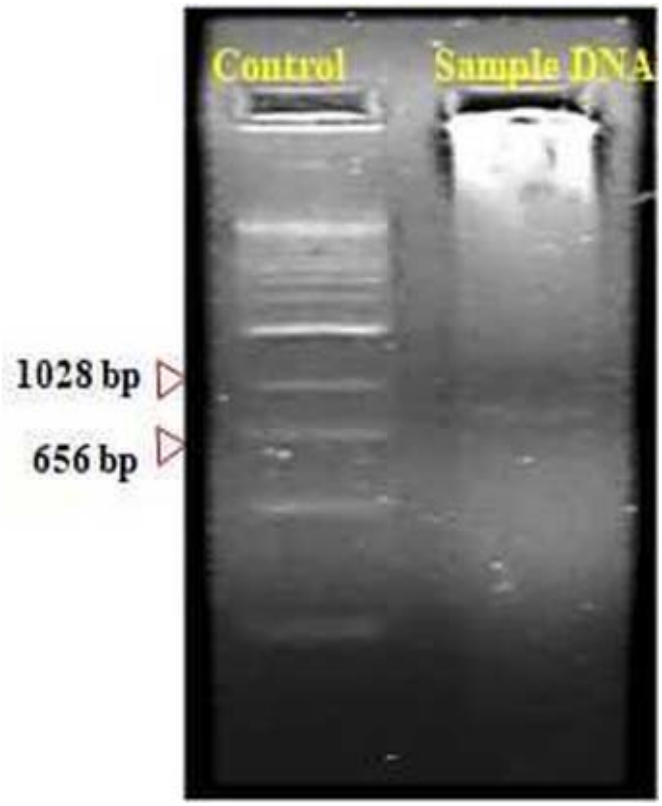

Figure 3. Agarose gel electrophoresis

PCR amplification of the 16S rRNA gene

The isolated DNA from the algal DNA sample was amplified for the 16S rRNA sequence by PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The PCR thermal conditions were set, for initial denaturation 95 ˚C for 30 sec (Denaturation at 95 ˚C for 30 sec, annealing 56 ˚C for 30-sec Extension at 72˚C for 30 sec for 35 cycles) and a final extension at 72 ˚C for 10 min followed by 4 ˚C hold. Figure 4 depicts the 16s rDNA PCR amplification.

ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare), is a one-tube enzymatic PCR cleanup reagent, which consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from PCR product mixture, is done before Sanger Sequencing so that there is no interference in downstream applications.

For every species of PCR amplified algal genome, five microliters of PCR product is mixed with 2 µl of ExoSAP-IT and incubated at 37°C for 30 min in a thermocycler for the rapid cleanup procedure, followed by enzyme inactivation at 80°C for 15 min followed by 4 ˚C.

Sequencing using BigDye Terminator v3.1

The sequencing reaction was performed in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator

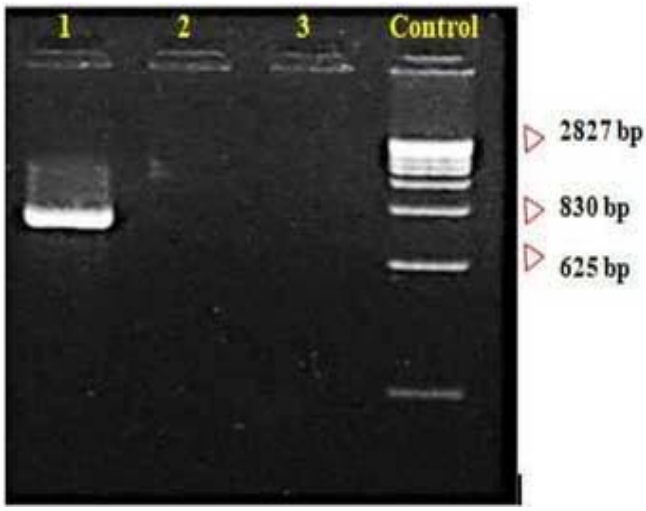

Figure 4. 16s rDNA PCR amplification

v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) following the manufacturer's protocol. This procedure was followed for every algal 16S rRNA gene and the 16S rRNA sequence was identified.

Culture and maintenance of *Microseira woelli*

Isolated *Microseira woelli* was inoculated in Erlenmeyer flask having BG11 medium and incubated at room temperature under continuous dark and sunlight period for 15-20 days, for their growth.

Lipid extraction

Total lipids were extracted from fresh microalgal biomass using a slightly modified method of Bligh and Dyer (Jensen 2008). The lipids were extracted with chloroform-methanol (2:1, v/v) and then separated into chloroform and aqueous methanol layers by the addition of methanol and water to give a final solvent ratio of chloroform: methanol: water of 1:1:0.9. The chloroform layer was washed with 20 mL of a 5% NaCl solution and evaporated to dryness. Thereafter, the weight of the crude lipid obtained from each sample was measured gravimetrically. Experiments were carried out in triplicate, and data were expressed as mean SD.

Biodiesel production

The extracted oil was evaporated in a vacuum to release hexane and ether. 0.25g NaOH was mixed with 24 ml methanol and stirred properly for 20 min. The mixture was poured in algal oil in a conical flask and shaker for 3 hrs by electric shaker at 300 rpm for the process of trans-esterification. The mixture was kept for 16h for the settling of biodiesel and sediment (Mathimani et al. 2018).

GC-MS Analysis

The GC-MS analysis was carried out for the algal species extract using a Clarus 500 Perkin- Elmer Gas Chromatograph equipped and coupled to a mass detector Turbo mass ver 5. 2.0 – Perking Elmer Turbomas 5.2 spectrometer with an Elite-(5%Phenyl 95% dimethyl polysiloxane), 30 m, 250 ìm capillary columns. The oven temperature was raised to 250°C, Injection port temperature was ensured as 280°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 1:10. The mass spectral scans range was set at 40-450 (MHz). Transfer line and source temperature was maintained at 200ºC and 250ºC respectively. Figure 5 shows the chromatogram of GC-MS analysis of *Microseira woelli* biodiesel.

RESULTS

The isolated algal samples were seeded in the BG-11 medium at 25°C for 5days. The pure colonies were isolated and viewed under a trinocular microscope

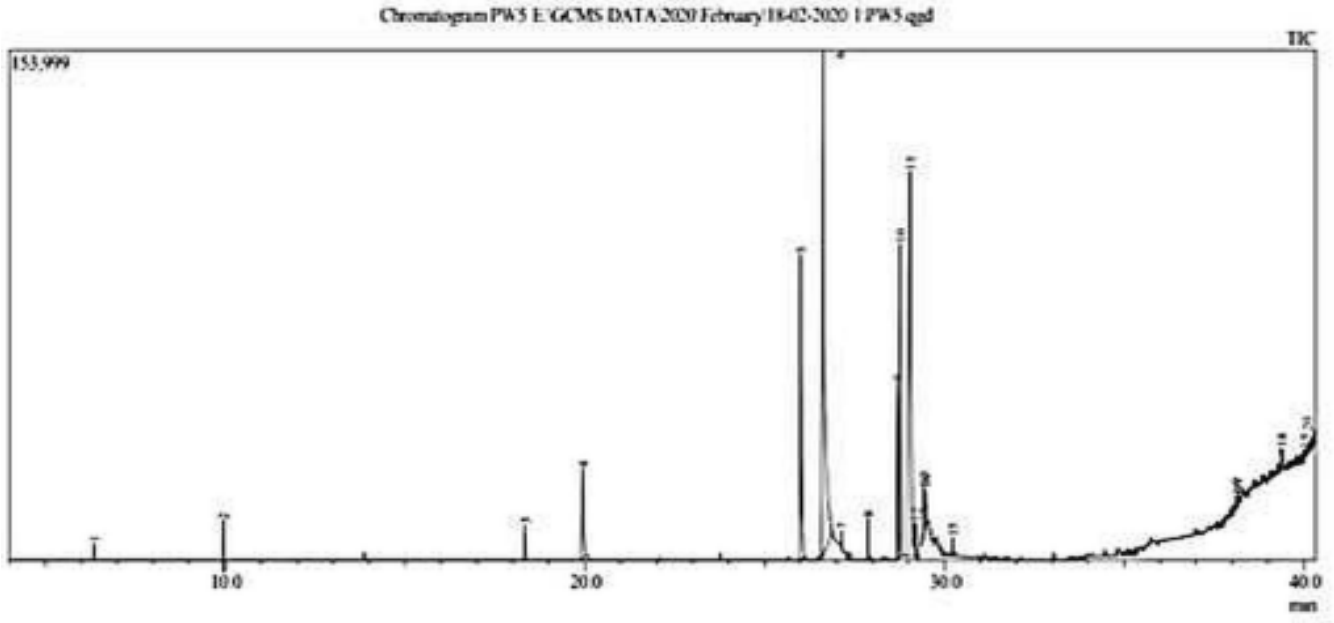

Figure 5. Chromatogram of GC-MS analysis of *Microseira woelli* biodiesel

(Labomed Vision 2000) by wet mounting method. The algae were found to be rod-shaped and belonged to the Cyanobacteria. The family was identified as Oscillatoriaceae with the help of Manual of Freshwater Algae of Tamil Nadu (Perumal and Anand. 2009). The algae were inoculated in BG-11 medium for the mass culture and incubated at dark and sunlight for 15-20 days. DNA isolation was carried out using the salting-out method and the extracted DNA was confirmed by 0.8 % agarose gel electrophoresis. The purity of DNA was further confirmed by UV spectrophotometer OD at 260 nm/ 280 nm and the OD value was found to be 1.8. PCR amplification was done using 16s rDNA primers under PCR conditions in a thermal cycler. The 1 % agarose gel electrophoresis results showed the amplified 16s rDNA PCR product was obtained in the size of 830 bp. The amplified PCR product was used for the sequencing of the 16s rDNA gene.

The sequence of the 16s rDNA gene having 830bp in length was analyzed in BLAST and the homological sequences were determined. The sequencing results were found to be highly similar to the sequence of ***Microseira woelli*** **NIES-4236**, 16s rRNA gene (**Accession ID: MK577503.1**) with 98.41% identity.

The *M. woelli* culture was subjected to lipid extraction using chloroform and methanol mixture in a separating funnel and the weight of the lipid obtained was measured to be 12.125 mg/mL. The algal oil was extracted and the catalyst was added. The mixture was kept in a shaker for the process of trans-esterification. The biodiesel thus obtained was analyzed in GC-MS. The present study revealed the greater amounts of alkyl ester compounds obtained from trans-esterification reaction. The GC-MS results show the presence of hexadecanoic acid-methyl ester, 8,11,14-docosatrienoic acid-methyl ester, 1,2-benzene dicarboxylic acid- diethyl ester at higher levels (Table 1).

Table 1. List of compounds present in *Microseira woelli* biodiesel

Compounds obtained from GC-MS
Fumaric acid, 2-methylpentyl tridecyl ester
Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester
2-propanamine, 2-methyl-n-[(4-nitrophenyl) methylene]-
1,2-benzenedicarboxylic acid, diethyl ester
Hexadecanoic acid, methyl ester
n-hexadecanoic acid
(1r,2r,4s,5s)-(-)-2-hydroxy-6-oxabicyclo[3.1.0]hexane-4-yl acetate
(z,z)-2,5-undecadien-1-ol
11,14-eicosadienoic acid, methyl ester
8,11,14-docosatrienoic acid, methyl ester
2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]-
Tetradecanoic acid, 12-methyl-, methyl ester
1,6-heptadiene-3-d, 5-methyl-
Bicyclo[3.1.0]hexan-2-one
9-tricosene, (e)- 6-chlorohexanoic acid, 2,3,4,6-tetrachlorophenyl ester
1,6-octadien-3-ol, 8-chloro-6-(chloromethyl)-2-methyl-, (z)-(-)-
2-pyrrolidinone, 5-[(3,4-dimethyl-1h-pyrrol-2-yl)methylene]-4-hydroxy-3,3-dimethyl-, (z)-(.+-.)-
(r)-(+)-1-(3-iodopropyl)dodecyl acetate
1-but-1-enylaziridine

DISCUSSION

Koley et al. (2018) and Saravanan et al. (2018) studied the biodiesel production from eco-friendly sources like vegetable oil/animal oil by the process of trans-esterification. Mathimani and Mallick (2019) and Saravanan et al. (2018) worked on production of biodiesel from third generation algal feedstocks from cyanobacteria, seaweed and diatoms and were found to be more feasible. Peng et al. (2008) reported the potential of using microbial oils in biodiesel production because of their structure and composition of fatty acid. The algae were found to accumulate lipids at more than 20% of their biomass. Research works showed the algal species possessed enough amounts of lipids and carbohydrates; they could be exploited for the production of biodiesel and bioethanol (Anto et al. 2020). The algae also holds many advantages like rapid growth rate, small usage of land and high amount of lipid content (Yang et al. 2011, Schenk et al. 2008). Chinnasamy et al. (2010) had achieved 6.82% of oil yield while

culturing a microalgae consortium in wastewater from carpet mills. Hossain et al. (2020) reported the mass culturing of cyanobacteria to obtain biomass for extracting total lipids. Fatty acid methyl ester (FAME) or biodiesel was produced from extracted lipid by *trans*-esterification reaction. The total lipid content was recorded highest in *Oscillatoria* sp (Hossain et al. 2020). This asserts our study of biodiesel production from *Oscillatoria* sp for better lipid extraction and biodiesel production. In this work, the oil content of nearly 18% was retrieved from *Microseira woelli* and about 70% of biomass was recovered. The trans-esterification process was accomplished to produce the biodiesel from the lipid content of cyanobacteria (Jensen 2008). Guan et al., 2011 has reported determination of biodiesel using gas chromatography–mass spectrometry (GC–MS) method qualitatively for seven free fatty acids and nine fatty acid methyl esters which were produced by wild-type of genetically engineered strain *Synechocystis* PCC 6803. Mathimani et al. (2018) extended their biodiesel analysis using Gas chromatography coupled mass spectrometry (GC-MS). Reports show that FAME (Fatty Acid Methyl Esters) component was identified using gas chromatography (GC) and FAME analysis showed cyanobacteria biodiesel contained linolelaidic acid methyl ester, Cis-8,11,14-eicosatrienoic acid methyl ester, Cis-10-heptadecanoic acid methyl ester, linolaidic acid methyl ester, Cis-9-oleic acid methyl ester, methyl arachidate and Cis-8,11,14-ecosatrieconoic acid methyl ester which revealed that cyanobacteria could use as potential source for biodiesel industry because of their high fatty acid content (Hossain et al. 2020). The biodiesel produced from *M. woelli* extract also exhibited greater amount of methyl/alkyl esters which confirmed the trans-esterification of lipids present in the algal species. Biodiesel was produced from different algal species like *Lyngbya majusula, Anabaena variabilis, Oscillatoria annae, Synechococcus elongatus* but no such work has been accomplished with *M. woelli* which entitles the novelty of the research work.

CONCLUSIONS

Biodiesel was successfully produced from the algal strain isolated from the fresh water ponds by lipid extraction followed by trans-esterification of fatty acids. Using PCR and sequencing of 16s rRNA gene, the novel strain was identified as *Microseira woelli* (Kennis 2017) which belonged to Oscillatoriaceae family and Cyanophyceae class. The biodiesel obtained from the extract was rich in fatty acid esters and confirmed by GC-MS analysis. Thus, the cyanobacteria are found to be the potential candidate for the production of biofuels. They also have additional properties that allow them to be used as ideal candidates for the development of eco-friendly systems for the generation of energy which might represent a simpler and cleaner system for the production of sustainable energy.

ACKNOWLEDGEMENT

The author thanks Trichy Research Institute of Biotechnology, J.J college of Arts and Science for providing lab facilities to carry out the research work. The corresponding author also thanks the co-author for guiding and motivating throughout the research work.

Authors' contribution: Both the authors contributed equally.

Conflict of interest: The authors declares no conflict of interest

REFERENCES

Anto, S., Mukherjee, S.S., Muthappa, T., Mathimani, R., Deviram, G., Kumar, S.S., Verma, T.N. and Pugazhendhi, A. 2020. Algae as green energy reserve: Technological outlook on biofuel production. Chemosphere, 242(12), 125079.

APHA (American Public Health Association). 1912. Standard methods for the examination of water and wastewater (Vol. 2). American Water Works Association, Water Pollution Control Federation and Water Environment Federation.

Bruno, L., di Pippo, F., Antonaroli, S., Gismondi, A., Valentini, C. and Albertans, P. 2012. Characterization of biofilm-forming cyanobacteria for biomass and lipid production. Journal of Applied Microbiology, 113, 1052–1064.

Chinnasamy, S., Bhatnagar, A., Hunt, R.W. and Das, K.C. 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel application. Bioresource Technology, 101, 3097-3105.

Falcon, L.I., Magallon, S. and Castillo, A. 2010. Dating the cyanobacterial ancestor of the chloroplast. ISME Journal,

4, 777–783.

Fischer, W.W., Hemp, J. and Johnson, J.E. 2016. Evolution of oxygenic photosynthesis. Annual Review of Earth Planet Science, 44, 647–683.

Glithero, N.J., Wilson, P. and Ramsden, S.J. 2015. Optimal combinable and dedicated energy crop scenarios for marginal land. Applied Energy, 147, 82–91.

Gnansounou, E. 2010. Production and use of lignocellulosic bioethanol in Europe: current situation and perspectives. Bioresource Technology, 101, 4842–4850.

Guan, W., Zhao, H., Lu, X., Wang, C., Yang, M. and Bai, F. 2011. Quantitative analysis of fatty-acid-based biofuels produced by wild-type and genetically engineered cyanobacteria by gas chromatography–mass spectrometry. Journal of Chromatography A, 45, 8289-8293.

Hossain, M.F., Ratnayake, R.R., Mahbub, S., Kumara, K.W. and Magana-Arachchi, D.N. 2020. Identification and culturing of cyanobacteria isolated from freshwater bodies of Sri Lanka for biodiesel production. Saudi Journal of Biological Sciences, 12, 100-108.

Jensen, S.K. 2008. Improved Bligh and Dyer extraction procedure. Lipid Technology, 20, 280-281.

Jorgensen, H., van Hecke, J., Zhang, H., Malik, P.L., Filby, C. and Schjoerring, J.K. 2018. Wheat as a dual crop for biorefining: straw quality parameters and their interactions with nitrogen supply in modern elite cultivars. GCB Bioenergy, 11, 400–415.

Kadam, K.L., Forrest, L.H. and Jacobson, W.A. 2000. Rice straw is a lignocellulosic resource: collection, processing, transportation, and environmental aspects. Biomass Bioenergy, 18, 369–389.

Kasting, J.F. 2013. What caused the rise of atmospheric O_2? Chemical Geology, 362, 13–25.

Kennis, A. 2017. Validation of the noxious cyanophytre *Microseira woelli* (Farlow ex Gomont) GB McGregor & Sendall (Oscillatoriaceae). Notulae Algarum, 43, 1-3.

Kim, S. and Dale, B.E. 2004. Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenergy, 26, 361–375.

Klutz, I., Wicke, B., Leemans, R. and Faaij, A. 2017. Sustainability constraints in determining European bioenergy potential: A review of existing studies and steps forward. Renewable and Sustainable Energy Reviews, 69, 719–734.

Koley, S., Khadase, M.S., Mathimani, T., Raheman, H. and Mallick, N. 2018. Catalytic and non-catalytic hydrothermal processing of *Scenedesmus obliquus* biomass for bio crude production – A sustainable energy perspective. Energy Conservation, 163, 212-217.

Mathimani, T. and Mallick, N. 2019. A review on the hydrothermal processing of microalgal biomass to bio-oil - Knowledge gaps and recent advances. Journal of cleaner production, 217, 69-84.

Mathimani, T. and Mallick, N. 2018. A comprehensive review on harvesting of microalgae for biodiesel - Key challenges and future directions. Renewable and Sustainable Energy Review, 91, 1103-1120.

Mazard S., Penesyan, A., Ostrowski, M., Paulsen, I.T. and Egan, S. 2016. Tiny microbes with a big impact: The role of Cyanobacteria and their metabolites in shaping our future. Marine Drugs, 14, 97-100.

Murata, N., Wada, H. and Gombos, Z. 1993. Modes of fatty acid desaturation in cyanobacteria. Plant Cell Physiology, 33, 933–941.

Nilsson, D. 2000. Dynamic simulation of straw harvesting systems: influence of climatic, geographical, and biological factors on performance and costs. Journal of Agricultural Engineering and Research, 76, 27–36.

Panoutsou, C., Perakis, C., Elbersen, B., Zheliezna, T. and Staritsky, I. 2017. Assessing potentials for agricultural residues. pp 169-197. In: Panoutsou, C. (Ed.) Modeling and Optimization of Biomass Supply Chains. Academic Press.

Passoth, V. 2014. Molecular mechanisms in yeast carbon metabolism: bioethanol and other biofuels. Pp. 217-259. In: Piskur, J. and Compagno, C. (Eds.) Molecular mechanisms in yeast carbon metabolism Springer, Berlin, Heidelberg.

Passoth, V., Tabassum, M.R., Nair, H.A,. Olstorpe, M., Tiukova, I. and Stahlberg, J. 2013. Enhanced ethanol production from wheat straw by integrated storage and pre-treatment (ISP). Enzyme and Microbial Technology, 52, 105–110.

Peng, X. and Chen, Y. 2008. Single cell oil production in solid-state fermentation by *Microsphaeropsis* sp. from steam-exploded wheat straw mixed with wheat bran. Bioresource Technology, 99, 3885-3889.

Perumal, G.M. and Anand, N. 2009. Manual of Fresh water Algae of Tamil Nadu. Centre for Advanced studies in Botany, University of Madras, Guindy campus, Chennai. 116 pp.

Rittmann, B.E. 2008. Opportunities for renewable bioenergy using microorganisms. Biotechnology and Bioengineering, 2, 203–212.

Saravanan, A.P., Mathimani, T., Deviram, G., Rajendran, K. and Pugazhendhi, A. 2018. Biofuel policy in India: A review of policy barriers in sustainable marketing of biofuel. Journal of Cleaner Production, 193, 734-747.

Schenk, P.M., ThomasHall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H. and Posten, C. 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Resource, 1, 20-43.

Schirrmeister, B.E., Sanchez-Baracaldo, P. and Wacey, D. 2016. Cyanobacterial evolution during the Precambrian. International Journal of Astrobiology, 15, 187–204.

Sims, R.E.H., Mabee, W., Saddler, J.N. and Taylor, M. 2010. An overview of second-generation biofuel technologies. Bioresource Technology, 101, 1570–1580.

Talebnia, F., Karakashev, D. and Angelidaki, I. 2010. Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis, and fermentation. Bioresource Technology, 101, 4744–4753.

Tishler, Y., Samach, A., Rogachev, I., Elbaum, R. and Levy, A.A. 2015. Analysis of wheat straw biodiversity for use as a feedstock for biofuel production. BioEnergy Research, 8, 1831–1839.

Townsend, T.J., Sparkes, D.L. and Wilson, P. 2017. Food and bioenergy: Reviewing the potential of dual-purpose wheat crops. GCB Bioenergy, 9, 525–540.

Valentine, J., Clifton-Brown, J., Hastings, A., Robson, P., Allison, G. and Smith, P. 2012. Food vs. fuel: the use of land for lignocellulosic 'next generation' energy crops that minimize competition with primary food production. GCB Bioenergy, 4, 1–19.

Yang, J., Xu, M., Zhang, M., Hu, M. and Sommerfeld, M. 2011. Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance. Bioresource Technology, 102, 159-165.

Received:11th July 2021
Accepted:15th September 2021