Malaysian Journal of Microbiology, Vol 18(4) 2022, pp. 380-388
DOI: http://dx.doi.org/10.21161/mjm.221430

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Biofuel production potential of indigenous isolates of *Scenedesmus* sp. from lake water in Pakistan

Muhammad Imran Najeeb[1]*, Mansur-ud-Din Ahmad[2], Aftab Ahmad Anjum[1], Azhar Maqbool[3], Muhammad Asad Ali[1], Muhammad Nawaz[1], Tehreem Ali[1] and Rabia Manzoor[1]

[1]Institute of Microbiology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan.
[2]Department of Epidemiology and Public Health, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan.
[3]Department of Parasitology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan.
Email: imran.najeeb@uvas.edu.pk

Received 7 February 2022; Received in revised form 29 May 2022; Accepted 1 June 2022

ABSTRACT

Aims: This paper presents the report on biodiesel and biogas production at a laboratory scale from *Scenedesmus* strain.
Methodology and results: Previously isolated and identified *Scenedesmus* were grown in 10 Liter flask using BG-11 media at 16 h light and 8 h dark cycle. Oven-dried biomass (20 g) from 16-day-old culture of *Scenedesmus* was finely grounded and subjected to lipids extraction by chloroform-methanol-NaCl mixture. Microalgal lipids (6 mL) were subjected to transesterification by using NaOH leading to the production of 5 mL biodiesel and 4 mL of glycerin. Biodiesel was rich in methyl esters of linoleic acid, phosphorothioc acid and dodecanoic acid, as shown by gas chromatography-mass spectrometry (GC-MS) analysis. Oven-dried microalgae (2 g) without lipid extraction and leftover biomass (2 g) after lipid extraction were subject to biogas production through anaerobic digestion. Biogas (34, 27 and 19 mL) were recorded respectively in oven-dried whole biomass; lipid extracted biomass and control over a period of 15 days of anaerobic digestion.
Conclusion, significance and impact of study: It was concluded that water bodies are rich in diverse algae, especially *Scenedesmus* sp., and this algae can be cultured to produce biodiesel and biogas. But the lipid accumulation potential of microalgae requires special treatment and lipid extraction methods are not up to the mark, which is a major bottleneck in biofuel production from microalgae.

Keywords: Biogas, biodiesel, biomass, microalgae, *Scenedesmus*

INTRODUCTION

Microalgae are either prokaryotic or eukaryotic organisms; being photosynthetic, they are able to fix carbon dioxide and water into energy by utilizing sunlight through organelles like chloroplast and chlorophyll a and b (Pröschold and Leliaert, 2007). These are fast-growing organisms and continue to grow in harsh conditions as well. The important prokaryotic microalgae are *Cyanobacteria* (Cyanophyceae), whereas eukaryotic microalgae are *Chlorophyta* (*Scenedesmus*) and *Diatoms* Microalgae can grow in diverse environmental conditions, including freshwater, brackish water and wastewater (Arora *et al.*, 2019).

Various microalgal genera such as *Scenedesmus*, *Chlorella*, *Dunaliella* and *Chlamydomonas* contain high carbohydrate and lipids contents (Li *et al.*, 2008). *Scenedesmus* can accumulate up to 60% carbohydrate and 36.9% lipid content, while *Chlorella vulgaris* can accumulate up to 37–55% carbohydrate and 29.8% lipid content in its dry biomass (Chen and Vaidyanathan, 2013). *Scenedesmus* being photosynthetic organisms, can be grown in the lab using photo-bioreactors supplemented with artificial light or in an open environment using sunlight. Various strategies have been employed for laboratory-scale and large-scale production of phototrophic algae. Large-scale indoor and outdoor production of *Scenedesmus* biomass is possible using highly controlled photo-bioreactors or fermenters indoors, while ponds, banks of rivers and streams are a source outdoors (Soares *et al.*, 2018).

Scenedesmus sp. can be used to obtain biofuels,

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 380-388
DOI: http://dx.doi.org/10.21161/mjm.221430

including biodiesel, syngas, biogas, bio-oil, bioethanol and biohydrogen (Dasgupta *et al.*, 2018). Mechanical and chemical methods are being used for lipid extraction. Chemical extraction of lipids from microalgae is possible by using solvent extraction, supercritical CO_2 extraction and ionic liquid extraction techniques. Several organic solvents like n-hexane, chloroform, methanol, ethanol, ether, dichloromethane, and isopropanol can be used to extract lipids (Mubarak *et al*., 2015).

Biodiesel has attracted attention as an alternative fuel in recent times, as it causes moderation of carbon dioxide and also acts as an alternative for petroleum. Biodiesel is a mono-alkyl ester of long-chain fatty acids derived from vegetable oil, plant oil, microalgal oils and animal fats (Mofijur *et al*., 2019). Methane and carbon dioxide are the main components of biogas. Biogas is produced in the presence of methanogenic bacteria by anaerobic digestion of organic matter. Electricity, fuel cells, liquid fuel and various other applications are produced from biogas. For biogas production, microalgal species of freshwater, marine algae and cyanobacteria can act as alternative organic sources (Zabed *et al*., 2020). *Scenedesmus* can also be used for biogas production as they contain no lignin and less cellulose, therefore demonstrating good process stability and high conversion efficiency for biogas production compared to waste organic sources and plant material (Harun *et al.*, 2011). The current study was designed to explore the bio-energy production potential of indigenous microalgae. In this study, we isolated and characterized the indigenous microalgae *Scenedesmus* as a potential candidate for biofuel production under local conditions.

MATERIALS AND METHODS

Isolation and identification

Water sample (10 mL) having a green tinge each from two different locations of Jallo Park Lake, Lahore, Pakistan, was collected in the sterile water container and transported to Postgraduate Research Lab, Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore for further analysis. Blue Green-11 (BG-11) medium was used for isolation, growth and maintenance of *Scenedesmus* (Andersen and Kawachi, 2005). For isolation, water (1 mL) was diluted 10-folds in BG-11 broth and 1 mL from each dilution was spread on BG-11 agar plates and incubated under 1000 lux light (16 h light and 8 h dark cycle) at room temperature till the presence of colonies. The colonies having a difference in shape and size of cells were re-streaked on BG-11 agar plates. Cells from purified colonies were observed under a light microscope (Nikon, Japan) at 400× for identification.

Preliminary lipid analysis

Preliminary analysis for the presence of lipids was done by Nile red staining assay (Rattanapoltee and Kaewkannetra, 2013). After staining, cells were observed under a fluorescent microscope (Optic 2x2F-EFD3, Nikon, Japan) at 400× using 490 excitation and 580 emission filter and results were recorded.

Preparation of seed culture

For seed culture, a single purified colony of selected *Scenedesmus* was picked from BG-11 agar plates and inoculated in 1000 mL BG-11 broth having pH 7.5. The flask containing inoculum was incubated at room temperature (25 °C), supplied with 1000 lux light (16 h light and 8 h dark cycle) and bubbled with filtered fresh air at 1 bubble/sec for 16 days. This growth was used as seed culture for further biomass production. For pilot scale growth, a glass carboy containing 20 L sterilized BG-11 broth at pH 7.5 was inoculated with the seed culture (1000 mL) and incubated at room temperature (25 °C) at 1000 lux light (16 h light and 8 h dark cycle), bubbled with filtered fresh air at 1 bubble per sec for a period of 16 days. The growth of seed culture and in carboy was measured at 680 nm using an enzyme-linked immunosorbent (ELISA) reader (Rayto, RT-2100C Microplate reader).

Harvesting of biomass

After 16 days of incubation, the air supply was shut to settle down microalgae. The settled microalgae (post 24 h) were collected by decanting 2/3rd of the growth media carefully so as not to disturb the sedimented growth, followed by centrifugation at 10,000 rpm for 10 min. The pellet was oven-dried in a crucible at 70 °C for 2-3 days.

Biochemical profile

Biochemical quantification of lipids was done according to a colorimetric sulfo-phospho-vanillin assay (Cheng *et al*., 2011). At the same time, carbohydrate and protein were quantified through Anthrone assay and Bradford assay, respectively (Chen *et al.*, 2017).

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis of *Scenedesmus* was conducted following (Dean *et al.*, 2010). To determine constituents of *Scenedesmus*, fifteen days old culture (1.5 mL) was centrifuged at 6,000 rpm. The supernatant was discarded, and the pellet was washed with normal saline thrice to remove the salts. The pellet was oven-dried at 70 °C and used for FTIR scan using the FTIR system (Agilent Technologies Cary 630).

Extraction of lipids

Oven-dried biomass (20 g) of *Scenedesmus* was used for the extraction of lipids. Lipids were extracted from biomass by solvent extraction chloroform-methanol-NaCl (1:2:1 v/v) with the samples in a proportion of 1:1 (Axelsson and Gentili, 2014).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 380-388
DOI: http://dx.doi.org/10.21161/mjm.221430

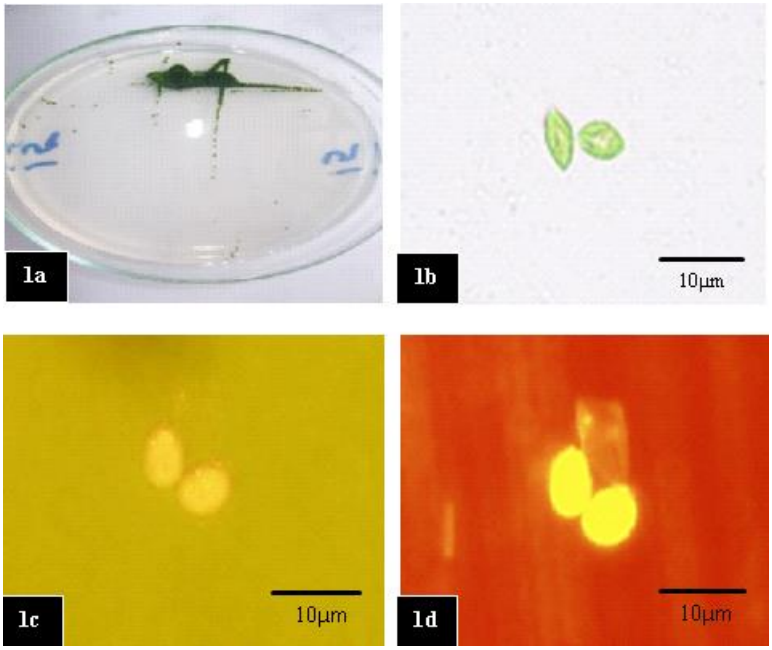

Figure 1: 1a: Pure colonies of *Scenedesmus* at BG-11 agar. 1b: Microscopic view (400×) of *Scenedesmus* cells. 1c: Fluorescent microscopic view of *Scenedesmus* cells after Nile red staining under yellow filter (490 excitation and 580 emission) at 400×. 1d: Fluorescent microscopic view of *Scenedesmus* cells after Nile red staining under red filter (490 excitation and 580 emission) at 400×. Presence of yellow fluorescence in both yellow and red filters is indicative of triglycerides.

Biodiesel production

Lipids extracted from biomass were converted into biodiesel using a trans-esterification protocol (Fukuda *et al*., 2001). Extracted lipids were transferred to a falcon tube, and chemical grade pure methanol and NaOH were added in known proportion (3:1:0.5). The mixture was incubated at 60 °C for 30 min. The quantity of recovered methyl ester (biodiesel) and glycerol were recorded.

GC-MS analysis

Methyl ester produced after trans-esterification was also analyzed by GC-MS (Mishra *et al*., 2014). The CARBOWAX capillary column was used, with helium as carrier gas and injector heated at 260 °C. A volume of 1 mL trans-esterified product was passed through Whatman filter paper followed by a 0.45 µm syringe filter and later analyzed by GC-MS (Agilent Technologies, USA 6890N, 5975 MS/LCD mass selective detector using Spectral library, NIST).

Production of biogas

Biomass (4 g) of *Scenedesmus* (whole dried biomass and biomass after lipid extraction each) was subject to anaerobic digestion for the production of methane gas (Passos *et al*., 2013).

Anaerobic digestion was carried in 250 mL flasks; biogas produced was measured and collected underwater in an inverted graduated cylinder. The anaerobic reaction mixture (slurry) was formed by mixing 10% cattle dung (fresh) in 200 mL of normal saline. Later on, 2g *Scenedesmus* dried biomass and 2 g biomass after extraction of lipids were added in two separate flasks containing 10% cattle dung slurry; a negative control flask of 10% slurry without the addition of biomass was also prepared for analysis. In order to produce anaerobic conditions, these flasks were capped with a rubber stopper having a central hole from which an air tube is passed (to collect gas) to an inverted measuring cylinder in a 1000 mL beaker containing water. The rubber stopper was completely air-tightened by applying silicon around it. The whole setup was placed in an incubator at 37 °C for 15 days. The quantity of biogas produced (mL) was recorded as the displacement of water from the graduated cylinder (Ramos-Suárez *et al*., 2014).

RESULTS

Isolation and identification

Serial dilution along with the spread plate method was successful for the isolation of microalgal colonies. Petri plates were analyzed under Stereomicroscope at 200× and in total (n=20) no of colonies was selected on the bases of difference in color, shape and texture. Out of 20 colonies, colonies (n=5) having cells in the shape of ovoid to fusiform were selected and re-streaked on BG-11 agar plates (Figure 1a). *Scenedesmus* were identified

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 380-388
DOI: http://dx.doi.org/10.21161/mjm.221430

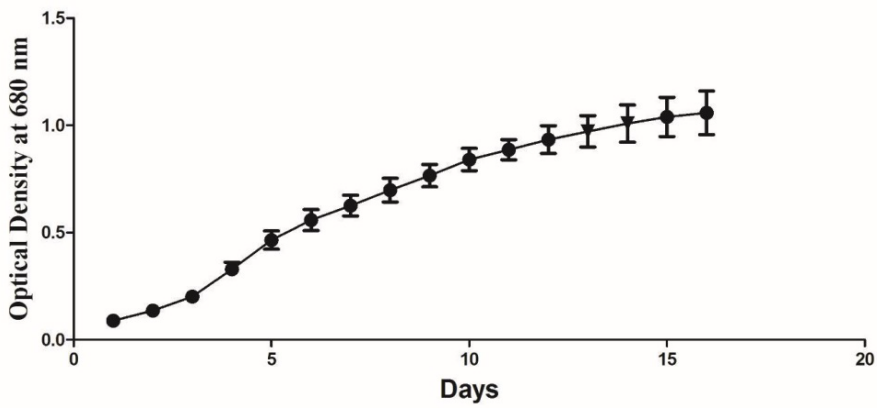

Figure 2: Growth response of *Scenedesmus* grown in BG-11 broth (pH 7.5, Temp 25 °C, light intensity of 1000× for 16 h light and 8 h dark) for 16 days as determined by measuring optical density at 680 nm.

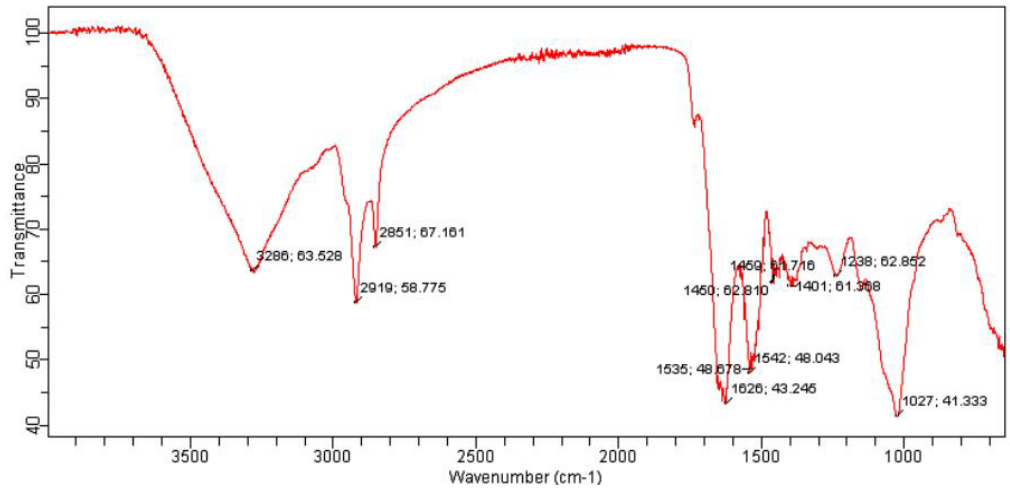

Figure 3: FTIR spectrum of *Scenedesmus*.

microscopically by viewing at 400× under a light microscope having cell shape of ovoid to fusiform, one pyrenoid and smooth or spined surface (Figure 1b).

Preliminary lipid analysis

Upon examination of selected colonies (n=5) by Nile red fluorescence dye, single colonies having better visual fluorescence were selected. Fluorescence of cells at 400× under yellow and red (490 excitation and 580 emissions) filter is represented in Figure 1c and Figure 1d. The presence of yellow fluorescence is indicative of the presence of lipids.

Measurement of growth and biomass

The optical density recorded for seed culture from day 1-16 was from 0.08 to 1.12 while O.D. The optical density for growth in 20 L round-bottom glass flask from day 1-16 was measured from 0.09 to 0.995. The growth curve is represented in Figure 2. The *Scenedesmus* biomass (23 g) after 16 days of growth was obtained after drying from a 20 L round-bottom glass flask.

Biochemical profile and Fourier-transform infrared spectroscopy (FTIR) analysis

The biochemical profile of selected *Scenedesmus* was recorded as carbohydrate (55.58%), protein (19.09%) and lipids (13.2%) dry weight, respectively.

FTIR scan of *Scenedesmus* selected is shown in Figure 3. The peak 3285 cm^{-1} in the spectral range of 3029-3639 cm^{-1}, the peak 2919 cm^{-1} and 2851 cm^{-1} in the spectral range of 3012-2809 cm^{-1}, the peak 1626 cm^{-1} in the spectral range of 1587-1709 cm^{-1}, the peak 1535 cm^{-1}, 1542 cm^{-1} and 1450 cm^{-1} in the spectral area of 1583-1709 cm^{-1} and the peak 1027 cm^{-1} in the spectral range of 980-1072 cm^{-1} indicate the protein stretching bonds or amide A bonds, stretching of lipids and carbohydrates, proteinamide-1 bonds, presence of carbonyl stretches which indicates the presence of fatty acids and polysaccharide in carbohydrates, respectively.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(4) 2022, pp. 380-388
DOI: http://dx.doi.org/10.21161/mjm.221430

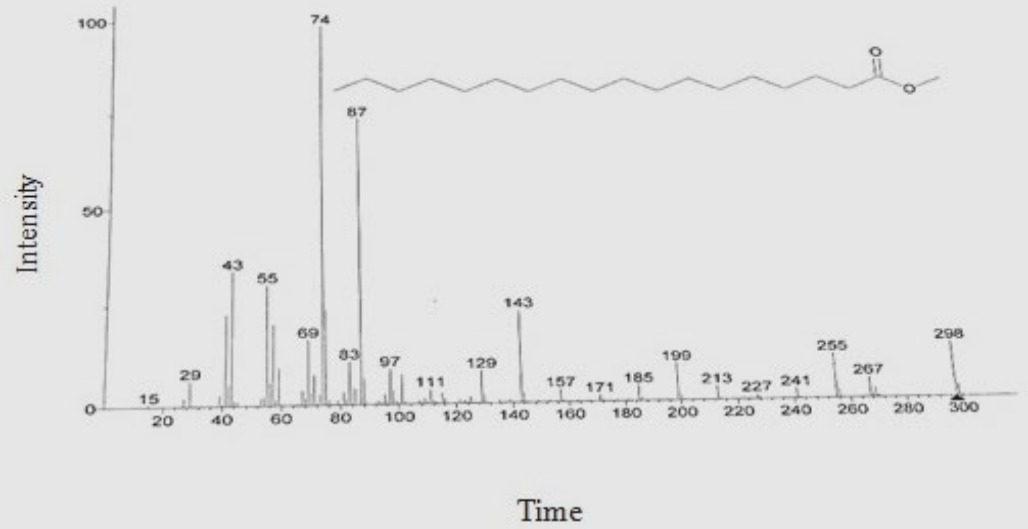

Figure 4: GC-MS spectrum of methyl esters produced by transesterification of extracted lipids from *Scenedesmus*.

Extraction of lipids and biofuel production

Dried biomass (20 g) of *Scenedesmus* was used for biodiesel production. A total of 2.5 mL of micro-algal lipids were extracted from 20 g of biomass. Upon transesterification of lipids with methanol in the presence of NaOH, 3 mL reaction mixture was obtained. Out of 3 mL reaction mixture, 2 mL of biodiesel and 1 mL of glycerin were obtained.

GC-MS analysis

The GC-MS analysis revealed the presence of methyl esters, including linoleic acid (53.6%), phosphorothioc acid (28.2%), dodecanoic acid (10.5%), octadecanoic acid (7.8%), heptadecanoic acid (3.5%) and hexadecanoic acid (2.2%). GC-MS profile of resultant biodiesel is shown in Figure 4.

Biogas production

Biogas was produced from 2 g of dried biomass and 2 g of leftover biomass after extraction of lipids in a lab-scale setup. The highest biogas (34 mL) was produced in a flask inoculated with whole dried biomass of *Scenedesmus* (2 g without lipid extraction) within the duration of 15 days.

The biogas started to produce on day 3 and achieved maximum production of 14 mL on day 12. The second highest production (27 mL) was recorded in a flask treated with leftover biomass after extraction of lipids. In total, 27 mL of biogas was produced in the course of 15 days. Maximum production 12 mL was observed on the 12th day. Minimum gas was produced in control over 15 days process. In total, 19 mL of biogas was produced with a production maximum on the 9th day (10 mL). Data is shown in Figure 5.

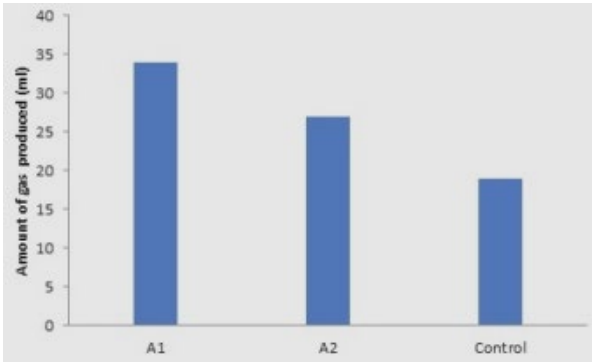

Figure 5: Production of biogas by anaerobic digestion using *Scenedesmus* (4 g). A1: Biogas (34 mL) produced by dried biomass of *Scenedesmus* (2 g) after being subjected to anaerobic digestion with 10% cattle dung slurry for 15 days. A2: Biogas (27 mL) produced by dried biomass (2g) of *Scenedesmus* after extraction of lipids subjected to anaerobic digestion with 10% cattle dung slurry for 15 days. Control: Biogas (19 mL) produced by 10% cattle dung slurry during 15 days of anaerobic digestion.

DISCUSSION

The current research was focused on the assessment of the bioenergy production potential of indigenous *Scenedesmus.* In our previous study (unpublished data), the microalgae (*Scenedesmus*) represented the highest lipid accumulation potential which in result will lead to higher production of biofuel. Makarevičienė *et al*. (2011) cultivated the *Chlorella* and *Scenedesmus* isolated from water samples in the laboratory and these studies indicated that algal species can be isolated and cultivated in lab-scale cultures and are ubiquitous in nature. Microalgae can be preliminarily identified by microscopy

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

(Abdelaziz *et al*., 2014). Microscopic characteristics for identification of microalgae include unicellular or multicellular, occurring singly or in the form of clusters, the shape may be circular, elliptical or elongated, pyrenoids may be present, chloroplast is cup-shaped, circular or griddle shaped, mucilaginous sheet present or absent (Selvarajan *et al*., 2015). Out of 10 isolates obtained, only 5 isolates were selected as *Scenedesmus*. Almost all selected isolates belong to class Chlorophyceae, which includes *Chlorella*, *Scenedesmus*, *Dunaliella*, *Botyrococcus*, *Ankistrodesmus*, *Graesiella*, *Chroococcus*, *Selenastrum* and *Haematococcus* (van den Hoek *et al*., 1995). Selection criteria for *Scenedesmus* microalgae for further studies were the presence of fluorescence (Nile red assay for lipids), Nile red dye binds with the lipids (triglycerides) and upon excitation at 490 nm and emission at 580 nm this dye emits yellow fluorescence for lipids and red fluorescence for chlorophyll. The Nile red staining presents a linear relationship with lipids present in microalgae (Cheng *et al*., 2021). Bark and Narula (2014) also followed the same criteria for the selection of microalgae for further studies.

Biochemically microalgae are rich in carbohydrates, proteins, lipids and other ingredients, including dietary fiber, ash, carotenoids and moisture (Niccolai *et al*., 2019). Micro-algal contents of carbohydrate, lipids and proteins vary according to climate change, nutrition availability and stress factors. Secondly, the detection methods used for the analysis also have limitations (Gonçalves *et al*., 2019). *Scenesdesmus* strain used in the study contains 55.85% carbohydrate, 19.09% proteins and 13.2% lipids; the rest material may contain ash, dietary fiber, carotenes and moisture contents. The methods used for biochemical analysis also have limiting factors like they require proper extraction of compounds and later detection, which may lead to errors in estimation (El Zokm *et al.*, 2021).

FTIR spectrum of *Scenedesmus* showed a peak 3285 cm^{-1} which is indicative of protein stretching bonds and other studies also reported that protein stretching bonds of *Scenesdesmus obliquus* is at 3330 cm^{-1} (Duygu *et al*., 2012). In our study peak for fatty acids were found at 1535, 1542 and 1450 cm^{-1}, while other studies about *Scenesdesmus* showed peak of 1789, 1648 and 1484 cm^{-1} for esters and bands in the range of 1700 to 1800 cm^{-1} (carbonyl stretch), which indicates the presence of fatty acids (Sudhakar and Premalatha, 2015).

Large-scale production of biofuels from microalgae faces potential problems, one of which is the extraction of lipids from microalgae. The lipid extraction step for the production of biofuel from microalgae faces high costs and is less efficient; a decrease in extraction efficiency leads to higher prices of algal fuels. Chloroform, n-hexane and methanol are used as lipids solvent, but the lipid yields were less as compared to a combination of chloroform/methanol and chloroform/methanol/water. This observation is also supported by other researchers that single solvent systems are less potent in extracting lipids from microalgae (Axelsson and Gentili, 2014; Shin *et al*., 2018). In order to improve the lipid extraction from microalgae, combinations of solvents were used as chloroform/methanol/NaCl (Li *et al.*, 2014). Axelsson and Gentili (2014) also reported that the chloroform/methanol/NaCl mixture extracts more lipids from microalgae than other solvent mixtures.

Biodiesel or fatty-acid methyl esters are important products obtained from lipids of algal biomass. Triglycerides (lipids) react with alcohol in the presence of a catalyst (alkali) to produce fatty acid methyl esters and glycerol (Chisti, 2007). Production of biodiesel from microalgae by transesterification is reported in the literature (Hossain *et al*., 2008; Halim *et al*., 2011; Ahmad *et al*., 2013). Dried biomass of *Scenedesmus* yields 2.5 mL lipids which, upon transesterification, produced 2 mL biodiesel and one mL glycerin. Other researchers also reported a similar observation with microalgae like *Spirogyra*, *Oedogonium* and *Scenesdesmus* (Hossain *et al*., 2008; Pugazhendhi *et al*., 2020). Another study also used sodium hydroxide and methanol as a catalyst to convert lipids obtained from *Chlorella*, *Scenesdesmus* to biodiesel and glycerol and also reported that when the extracted lipids were converted into biodiesel, only 6 mL (86%) biodiesel was obtained (El-Sheekh *et al*., 2018). In order to completely analyze the methyl ester formed after transesterification, the trans-esterified product (biodiesel) was subjected to GC-MS analysis. Usually, polar (phospholipids) and neutral lipids (triacylglycerol and cholesterol) are produced by microalgae, triacylglycerol being the more important lipids that are converted into biodiesel by a procedure of transesterification (Chisti, 2007). Fatty acids with a chain length of C:8 to C:20 are a suitable candidate to be converted into biodiesel (Fukuda *et al*., 2001; Kim *et al*., 2017). GC-MS analysis revealed the presence of methyl asters like linoleic acid, phosphorothioc acid, dodecanoic acid, octadecanoic acid methyl esters, heptadecanoic acid and hexadecadienoic acid. Other researchers also studied the GC-MS profile of *Scenedesmus,* and their findings are similar to our findings that biodiesel contains linoleic acid, palmitic acid and hexadecadienoic acid (Gouveia and Oliveira, 2009; Niemi *et al*., 2019).

Anaerobic digestion is a biological process that involves anaerobic microorganisms to break down the complex molecules (proteins, lipids, carbohydrates) into methane and carbon dioxide. In recent years, in order to scale up biodiesel production from microalgae and to make the process more energy-efficient, leftover biomass of microalgae after lipid extraction is being utilized in anaerobic digestion to produce biogas (Cavinato *et al*., 2017). In order to explore bioenergy potential, *Scenedesmus* were subjected to anaerobic digestion. The highest production of biogas (34 mL) was obtained from the whole dried biomass of *Scenedesmus* (2 g) in 15 days, while 27 mL of biogas were obtained from 2 g of biomass (after lipid extraction) in 15 days. Keymer *et al*. (2013) also obtained 212 mL methane from each gram of volatile solids and 140 mL methane from each gram of volatile solids after extraction of lipids from biomass with hexane. Ramos-Suárez and Carreras (2014) also obtained 272 mL methane per gram of volatile solids. Our

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

yield of biogas is low as compared to these studies, which may be due to the difference of biochemical profiles within the same species. The yield of biogas from micro-algal biomass (after extraction of lipids) is even low. This may be due to that chloroform inhibits the production of biogas (Yun *et al.*, 2014). Yun *et al.* (2016) also reported that there was a 30% decrease in methane production when dried microalgal biomass (after extraction of lipids by chloroform/methanol) was utilized for the anaerobic digestion. Mussgnug *et al*. (2010) also reported that species with high carbohydrate contents show less degradability in anaerobic digestion while species having proteins and lipids show more degradability.

CONCLUSION

It was concluded that water bodies are rich in diverse algae, especially *Scenedesmus* sp. and the algae can be cultured to produce biodiesel and biogas. But, the lipid accumulation potential of microalgae requires special treatment and lipid extraction methods are not up to the mark, which is a major bottleneck in biofuel production from microalgae. It is recommended that the lipid accumulation and biofuel production of selected *Scenedesmus* sp. may be further optimized by using different photo-bioreactors, photoperiods and various sources of nutrition like organic and inorganic nitrogen and phosphorous. For the mass-scale growth of algae, natural ponds and lakes may also be utilized using agriculture and industrial waste.

REFERENCES

Abdelaziz, A. E., Leite, G. B., Belhaj, M. A. and Hallenbeck, P. C. (2014). Screening microalgae native to Quebec for wastewater treatment and biodiesel production. *Bioresource Technology* **157, 140-148.**

Ahmad, F., Khan, A. U. and Yasar, A. (2013). Transesterification of oil extracted from different species of algae for biodiesel production. *African Journal of Environmental Science and Technology* **7(6), 358-364.**

Andersen, R. A. and Kawachi, M. (2005). Traditional microalgae isolation techniques. *In*: Algal Culturing Techniques. Andersen, R. A. (ed.). Academic Press, USA. **pp. 83-98.**

Arora, N., Laurens, L. M., Sweeney, N., Pruthi, V., Poluri, K. M. and Pienkos, P. T. (2019). Elucidating the unique physiological responses of halotolerant *Scenedesmus* sp. cultivated in sea water for biofuel production. *Algal Research* **37, 260-268.**

Axelsson, M. and Gentili, F. (2014). A single-step method for rapid extraction of total lipids from green microalgae. *PLoS ONE* **9(2), e89643.**

Bark, M. (2012). Cultivation of eleven different species of freshwater microalgae using simulated flue gas mimicking effluents from paper mills as carbon source. M.Sc. Thesis. Chalmers University of Technology, Gothenburg, Sweden.

Cavinato, C., Ugurlu, A., de Godos, I., Kendir, E. and Gonzalez-Fernandez, C. (2017). Biogas production from microalgae. *In:* Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End-products. Gonzalez-Fernandez, C. and Muñoz, R. (eds.). Woodhead Publishing, United Kingdom. **pp. 155-182.**

Chen, Y. and Vaidyanathan, S. (2013). Simultaneous assay of pigments, carbohydrates, proteins and lipids in microalgae. *Analytica Chimica Acta* **776, 31-40.**

Chen, Y., Xu, C. and Vaidyanathan, S. (2017). Microalgae: A robust "green bio-bridge" between energy and environment. *Critical Reviews in Biotechnology* **38(3), 351-368.**

Cheng, J., Du, X., Long, H., Zhang, H. and Ji, X. (2021). The effects of exogenous cerium on photosystem II as probed by *in vivo* chlorophyll fluorescence and lipid production of *Scenedesmus obliquus* XJ002. *Biotechnology and Applied Biochemistry* **68(6), 1216-1226.**

Cheng, Y. S., Zheng, Y. and VanderGheynst, J. S. (2011). Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. *Lipids* **46(1), 95-103.**

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology advances* **25(3), 294-306.**

Dasgupta, C. N., Nayaka, S., Toppo, K., Singh, A. K., Deshpande, U. and Mohapatra, A. (2018). Draft genome sequence and detailed characterization of biofuel production by oleaginous microalga *Scenedesmus quadricauda* LWG002611. *Biotechnology for Biofuels* **11, 308.**

Dean, A. P., Sigee, D. C., Estrada, B. and Pittman, J. K. (2010). Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae. *Bioresource Technology* **101(12), 4499-4507.**

Duygu, D. Y., Udoh, A. U., Ozer, T. B., Akbulut, A., Erkaya, I. A., Yildiz, K. and Guler, D. (2012). Fourier transform infrared (FTIR) spectroscopy for identification of *Chlorella vulgaris* Beijerinck 1890 and *Scenedesmus* obliquus (Turpin) Kützing 1833. *African Journal of Biotechnology* **11(16), 3817-3824.**

El-Sheekh, M., Abomohra, A. E., Eladel, H., Battah, M. and Mohammed, S. (2018). Screening of different species of *Scenedesmus* isolated from Egyptian freshwater habitats for biodiesel production. *Renewable Energy* **129, 114-120.**

El Zokm, G. M., Ismail, M. M. and El-Said, G. F. (2021). Halogen content relative to the chemical and biochemical composition of fifteen marine macro and micro algae: Nutritional value, energy supply, antioxidant potency, and health risk assessment. *Environmental Science and Pollution Research* **28(12), 14893-14908.**

Fukuda, H., Kondo, A. and Noda, H. (2001). Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bioengineering* **92(5), 405-416.**

Gonçalves, C. F., Menegol, T. and Rech, R. (2019). Biochemical composition of green microalgae

Pseudoneochloris marina grown under different temperature and light conditions. *Biocatalysis and Agricultural Biotechnology* **18, 101032.**

Gouveia, L. and Oliveira, A. C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology* **36(2), 269-274.**

Halim, R., Gladman, B., Danquah, M. K. and Webley, P. A. (2011). Oil extraction from microalgae for biodiesel production. *Bioresource Technology* **102(1), 178-185.**

Harun, R., Davidson, M., Doyle, M., Gopiraj, R., Danquah, M. and Forde, G. (2011). Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. *Biomass and Bioenergy* **35(1), 741-747.**

Hossain, A. S., Salleh, A., Boyce, A. N., Chowdhury, P. and Naqiuddin, M. (2008). Biodiesel fuel production from algae as renewable energy. *American Journal of Biochemistry and Biotechnology* **4(3), 250-254.**

Keymer, P., Ruffell, I., Pratt, S. and Lant, P. (2013). High pressure thermal hydrolysis as pre-treatment to increase the methane yield during anaerobic digestion of microalgae. *Bioresource Technology* **131, 128-133.**

Kim, M. G., Hwang, H. W., Nzioka, A. M. and Kim, Y. J. (2017). Enhanced lipid extraction from microalgae in biodiesel production. *Hemijska Industrija* **71(2), 167-174.**

Li, Y., Horsman, M., Wang, B., Wu, N. and Lan, C. Q. (2008). Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Applied Microbiology and Biotechnology* **81(4), 629-636.**

Li, Y., Naghdi, F. G., Garg, S., Adarme-Vega, T. C., Thurecht, K. J., Ghafor, W. A., Tannock, S. and Schenk, P. M. (2014). A comparative study: The impact of different lipid extraction methods on current microalgal lipid research. *Microbial Cell Factories* **13(1), 14.**

Makarevičienė, V., Andrulevičiūtė, V., Skorupskaitė, V. and Kasperovičienė, J. (2011). Cultivation of microalgae *Chlorella* sp. and *Scenedesmus* sp. as a potential biofuel feedstock. *Environmental Research, Engineering and Management* **3(57), 21-27.**

Mishra, S. K., Suh, W. I., Farooq, W., Moon, M., Shrivastav, A., Park, M. S. and Yang, J. W. (2014). Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. *Bioresource Technology* **155, 330-333.**

Mofijur, M., Rasul, M. G., Hassan, N. M. S. and Nabi, M. N. (2019). Recent development in the production of third generation biodiesel from microalgae. *Energy Procedia* **156, 53-58.**

Mubarak, M., Shaija, A. and Suchithra, T. V. (2015). A review on the extraction of lipid from microalgae for biodiesel production. *Algal Research* **7, 117-123.**

Mussgnug, J. H., Klassen, V., Schlüter, A. and Kruse, O. (2010). Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *Journal of Biotechnology* **150(1), 51-56.**

Narula, A. (2014). Biodiesel production from lipid generating microalgae. Ph.D. Thesis. CCS Haryana Agricultural University, Hisar, Haryana.

Niccolai, A., Zittelli, G. C., Rodolfi, L., Biondi, N. and Tredici, M. R. (2019). Microalgae of interest as food source: Biochemical composition and digestibility. *Algal Research* **42, 101617.**

Niemi, C., Lage, S. and Gentili, F. G. (2019). Comparisons of analysis of fatty acid methyl ester (FAME) of microalgae by chromatographic techniques. *Algal Research* **39, 101449.**

Passos, F., Solé, M., García, J. and Ferrer, I. (2013). Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment. *Applied Energy* **108, 168-175.**

Pröschold, T. and Leliaert, F. (2007). Systematics of the green algae: Conflict of classic and modern approaches. *In*: Unravelling the Algae: The Past, Present, and Future of Algal Systematics. Brodie, J. and Lewis, J. (eds.). CRC Press, Boca Raton. **pp. 123-153.**

Pugazhendhi, A., Arvindnarayan, S., Shobana, S., Dharmaraja, J., Vadivel, M., Atabani, A. E., Chang, S. W., Nguyen, D. D. and Kumar, G. (2020). Biodiesel from *Scenedesmus* species: Engine performance, emission characteristics, corrosion inhibition and bioanalysis. *Fuel* **276, 118074.**

Ramos-Suárez, J. L. and Carreras, N. (2014). Use of microalgae residues for biogas production. *Chemical Engineering Journal* **242, 86-95.**

Ramos-Suárez, J. L., Martínez, A. and Carreras, N. (2014). Optimization of the digestion process of *Scenedesmus* sp. *and Opuntia maxima* for biogas production. *Energy Conversion and Management* **88, 1263-1270.**

Rattanapoltee, P. and Kaewkannetra, P. (2013). Nile red, an alternative fluorescence method for quantification of neutral lipids in microalgae. *International Journal of Biotechnology and Bioengineering* **7(9), 889-893.**

Selvarajan, R., Felföldi, T., Tauber, T., Sanniyasi, E., Sibanda, T. and Tekere, M. (2015). Screening and evaluation of some green algal strains (Chlorophyceae) isolated from freshwater and soda lakes for biofuel production. *Energies* **8(7), 7502-7521.**

Shin, H. Y., Shim, S. H., Ryu, Y. J., Yang, J. H., Lim, S. M. and Lee, C. G. (2018). Lipid extraction from *Tetraselmis* sp. microalgae for biodiesel production using hexane-based solvent mixtures. *Biotechnology and Bioprocess Engineering* **23(1), 16-22.**

Soares, J., Loterio, R. K., Rosa, R. M., Santos, M. O., Nascimento, A. G., Santos, N. T., Williams, T. C. R., Nunes-Nesi, A. and Martins, M. A. (2018). *Scenedesmus* sp. cultivation using commercial-grade ammonium sources. *Annals of Microbiology* **68, 35-45.**

Sudhakar, K. and Premalatha, M. (2015). Characterization of micro algal biomass through FTIR/TGA/CHN analysis: Application to *Scenedesmus* sp. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* **37(21), 2330-2337.**

van den Hoek, C., Mann, D. G. and Jahns, H. M. (1995). Algae: An Introduction to Phycology. Cambridge University Press, United Kingdom.

Yun, H. S., Ji, M. K., Park, Y. T., Salama, E. and Choi, J. (2016). Microalga, *Acutodesmus obliquus* KGE 30 as a potential candidate for CO_2 mitigation and biodiesel production. *Environmental Science and Pollution Research* **23(17), 17831-17839.**

Yun, Y. M., Cho, S. K., Jung, K. W., Kim, M. S., Shin, H. S. and Kim, D. H. (2014). Inhibitory effect of chloroform on fermentative hydrogen and methane production from lipid-extracted microalgae. *International Journal of Hydrogen Energy* **39(33), 19256-19261.**

Zabed, H. M., Akter, S., Yun, J., Zhang, G., Zhang, Y. and Qi, X. (2020). Biogas from microalgae: Technologies, challenges and opportunities. *Renewable and Sustainable Energy Reviews* **117, 109503.**