BIOTROPIA ISSN: 0215-6334 | e-ISSN: 1907-770X
The Southeast Asian Journal of Tropical Biology
Vol. 32 No. 1, 2025: 87 - 99
DOI: 10.11598/btb.2024.32.1.2428

Research Article

FATTY ACID PROFILES OF MICROALGAE ISOLATES FROM THE WATERS OF ITS DORMITORY POND IN SURABAYA, INDONESIA

Alfiyyana Nurrahma Mawardani[1], Dini Ermavitalini[1,*], Triono Bagus Saputro[1], Farid Kamal Muzaki[1], Adi Setyo Purnomo[2], Hery Purnobasuki[3]

[1]Department of Biology, Faculty of Science and Data Analytics, Sepuluh Nopember Institute of Technology, Surabaya 60111, Indonesia.

[2]Department of Chemistry, Faculty of Science and Data Analytics, Sepuluh Nopember Institute of Technology, Surabaya 60111, Indonesia.

[3]Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya 60115, Indonesia.

ARTICLE HIGLIGHTS

- Microalgae from ITS Dormitory Pond were identified using morphological analysis.
- The study analyzed the fatty acid composition of four microalgae genera.
- *Dictyosphaerium* had the highest total fatty acid content.
- The findings suggest potential applications of these microalgae in biotechnology.
- Further research is needed to improve fatty acid production.

Article Information

Received : 14 January 2025
Revised : 8 March 2025
Accepted : 10 March 2025

***Corresponding author, e-mail:**
diniermavitalini80@gmail.com

ABSTRACT

Microalgae are a diverse group of autotrophic microorganisms classified into prokaryotic and eukaryotic groups. Their ability to thrive in various environmental conditions enables the identification of species with distinct fatty acid profiles. The absence of a microalgae culture collection at Sepuluh Nopember Institute of Technology (ITS) motivated the selection of the ITS Dormitory Pond as the research site. This study aimed to identify microalgae species present in the pond through morphological characterization and analyze their fatty acid profiles. Morphological identification was conducted using taxonomic references, while fatty acid composition was determined via GC-MS analysis. The results identified four genera: *Dictyosphaerium, Vitreochlamys, Desmodesmus,* and *Chlorella*. Their fatty acid profiles varied, comprising saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). *Dictyosphaerium* contained 17.57% SFA, 12.28% MUFA, and 0.33% PUFA, while *Vitreochlamys* had 10.83% SFA and 10.19% MUFA. *Desmodesmus* exhibited 14.19% SFA, 7.39% MUFA, and 4.14% PUFA, whereas *Chlorella* had 1.92% SFA and 2.67% PUFA. These findings highlight the potential of microalgae from ITS Dormitory Pond as a natural source of fatty acids. Further research is recommended to optimize culture conditions for enhancing commercially valuable fatty acid production.

Keywords: fatty acid profile, ITS dormitory pond, microalgae diversity, microalgae identification, microalgae isolation

INTRODUCTION

Microalgae are unicellular eukaryotic microorganisms, ranging in size from 2 to 50 µm, distributed across various phyla and capable of thriving in freshwater, brackish, or saline water. As photosynthetic organisms, they play a vital role in aquatic ecosystems, contributing to 40% of global photosynthesis and producing nearly half of Earth's oxygen. Their ability to grow photoautotrophically using CO_2 and light makes their cultivation simple and cost-effective, converting these inputs into biomass and oxygen (Elisabeth *et al.* 2021). Microalgae synthesize bioactive compounds, such as proteins, polysaccharides, and lipids, which have applications in pharmacology, medicine, cosmetics, aquaculture, energy, agriculture, and functional foods (Dolganyuk *et al.* 2020).

Indonesia's rich biodiversity, with an estimated 200,000 - 800,000 microalgal species and 50,000 identified species, provides vast potential for exploring microalgae as a source of fatty acids (Wu *et al.* 2021). The growth and biochemical composition of microalgae are influenced by environmental factors, such as temperature, light intensity, nutrient availability, CO_2 concentration, and pH (Lee *et al.* 2014). This diversity, combined

with varied habitat conditions, makes Indonesia an ideal location for studying microalgal fatty acid profiles. The ITS Dormitory Pond was selected as the research site due to the absence of a microalgal isolate culture collection at Sepuluh Nopember Institute of Technology (ITS) and its potential as a natural microalgae habitat influenced by moderate pollution due to student activities.

Domestic waste disposal contributes organic and inorganic materials, altering the pond's physical, chemical, and biological properties, which in turn affect microalgal diversity and lipid accumulation. Studying microalgae in this environment provides insight into how anthropogenic activities affect microalgal diversity and lipid accumulation, which is crucial for biotechnological applications (Mazidah *et al.* 2013).

Microalgal fatty acids have attracted increasing interest due to their commercial potential. Lipids constitute 1 - 40% of microalgal dry weight, with some species accumulating up to 85% under specific conditions (Chen *et al.* 2023). Studies have shown that *Prorocentrum donghaiense* contains 49.32% ± 1.99% lipids, while *Nannochloropsis* species can accumulate 37 - 60% of dry weight as lipids (Gui *et al.* 2021; Milano *et al.* 2016). Microalgae produce various fatty acids, including saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are widely used in nutraceuticals production and aquaculture (Abdelkarim *et al.* 2024).

Despite the known benefits of microalgal fatty acids, there is limited research on the fatty acid profiles of indigenous microalgae species, particularly those from artificial freshwater environments, such as dormitory ponds. Understanding the fatty acid composition of microalgal isolates from such habitats provides valuable insight into their metabolic capabilities and potential industrial applications. Furthermore, identifying species-specific fatty acid profiles can support strain selection for targeted biotechnological applications, including biodiesel production and functional food development (Shang *et al.* 2024). This study aimed to identify microalgae through morphological observation and analyze their fatty acid profiles from isolates collected in the ITS Dormitory Pond, Surabaya. The findings are expected to contribute to the development of microalgal bioresources for biofuels, pharmaceuticals, and functional foods, while also providing baseline data for future microalgal cultivation and environmental biotechnology research.

MATERIALS AND METHODS

Study Area

This research was conducted from June 2023 to July 2024. Water samples were taken from ITS Dormitory Pond (Fig. 1; Table 1). Samples were observed in the Bioscience and Plant Technology Laboratory, Department of Biology, Faculty of Science and Data Analytics, ITS, Surabaya. Microalgae fatty acid profile test was carried out in the Faculty of Pharmacy, Airlangga University, Surabaya.

Procedures

Preparation of Agar Medium

Microalgae were isolated using Bold's Basal Medium (BBM) agar medium following the protocol of Ardiansyah *et al.* (2018). The medium was sterilized using an autoclave at 121 °C and 1.5 atm pressure for 30 minutes. The agar medium was poured into Petri dishes, sealed with plastic wrap, and allowed to solidify before use (Lee *et al.* 2014).

Microalgae Sampling, Isolation, and Cultivation

Microalgae were sampled using an active method from two ponds, with five sampling points per pond. Water was filtered through a 35 µm plankton net, and the samples were stored in labeled bottles and transported to the laboratory (Saputro *et al.* 2019).

Subsequently, the water samples were diluted via serial dilution (10^{-1} to 10^{-3}) by transferring 1 mL of the sample sequentially into test tubes with 9 mL of distilled water (Chaidir *et al.* 2016). Purification of microalgae samples was conducted using the streak plate method. Petri dishes were incubated at 27 °C under 30 Watt fluorescent light for 7 - 14 days until microalgae grew. Isolates were further purified on agar to obtain homogeneous cultures. Pure cultures were transferred to a liquid medium in glass bottles for growth (Saputro *et al.* 2019).

Cultivation was conducted following the protocol of Kawaroe *et al.* (2010) by mixing 20% of microalgae culture with 80% of fresh liquid medium. Cultivation began at 20 mL and scaled

to 5 L in media with pH 6, temperature 27 °C, light intensity 30 Watts, and continuous aeration (Kawaroe *et al.* 2010). Cell growth was monitored daily by measuring optical density at 680 nm (Lee *et al.* 2014). Harvesting occurred during the early stationary phase, identified via the growth curve, to maximize metabolic compound accumulation, including lipids. Microalgae were separated from the medium using high-speed centrifugation (Chen *et al.* 2011; Ferreira *et al.* 2019).

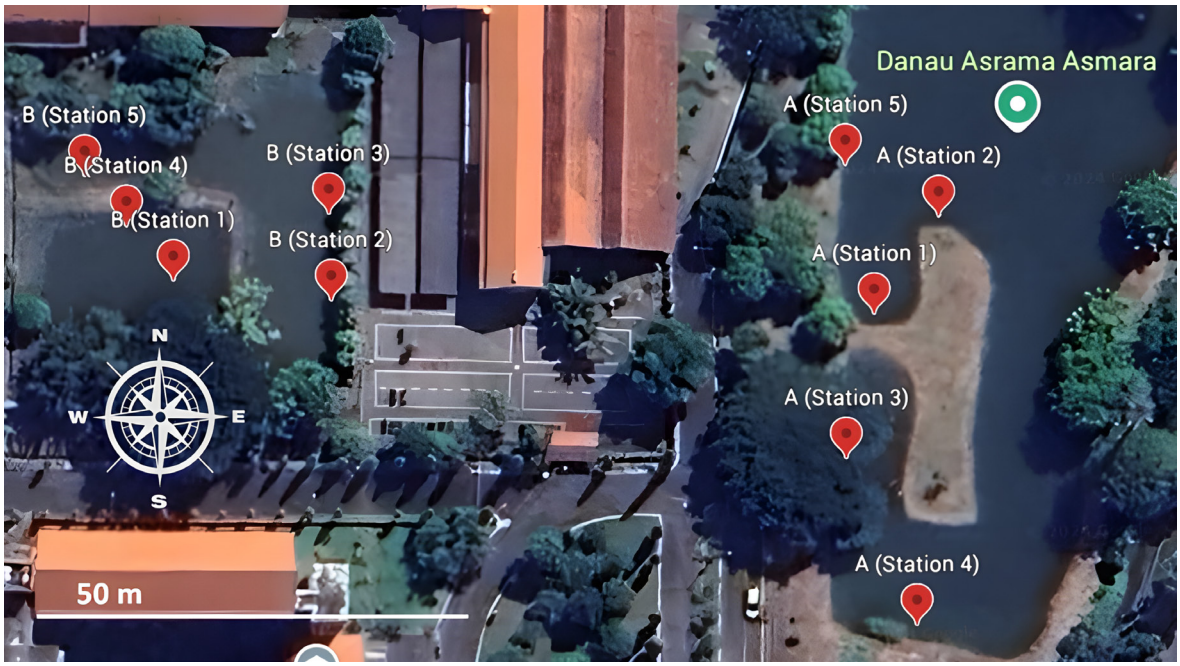


Figure 1 The location of microalgae sampling in ITS Dormitory Pond, Surabaya, Indonesia
Notes: Pond A is at the front of the dormitory; Pond B is behind the dormitory.

Table 1 Sampling stations coordinates for microalgae collection at the ITS Dormitory Pond

No.	Station	Coordinate	
1.	Pond A (Station 1)	7°17'17"S	112°47'32"E
2.	Pond A (Station 2)	7°17'17"S	112°47'32"E
3.	Pond A (Station 3)	7°17'18"S	112°47'32"E
4.	Pond A (Station 4)	7°17'19"S	112°47'32"E
5.	Pond A (Station 5)	7°17'17"S	112°47'32"E
6.	Pond B (Station 1)	7°17'17"S	112°47'29"E
7.	Pond B (Station 2)	7°17'17"S	112°47'30"E
8.	Pond B (Station 3)	7°17'17"S	112°47'30"E
9.	Pond B (Station 4)	7°17'17"S	112°47'29"E
10.	Pond B (Station 5)	7°17'17"S	112°47'29"E

Morphological Identification

Morphological identification of isolated microalgae was done microscopically (Chaidir *et al.* 2016). Samples were placed on a glass slide, added with distilled water, and observed under an Olympus BX71 microscope with 1,000x magnification and immersion oil (Salindeho *et al.* 2022). Identification was based on characteristics, such as cell color, shape, size, and the presence of flagella and pyrenoids, using the books titled *"Freshwater Algae: Identification, Enumeration and Use as Bioindicators"* (Bellinger & Sigee 2015) and *"Freshwater Algae of North America"* (Wehr *et al.* 2015).

Microalgae Fatty Acid Profile Test

The fatty acid profile of microalgae was analyzed using GC-MS, following these steps: 1) fatty acid extraction, 2) fatty acid derivatization, and 3) GC-MS analysis (Chiu & Kuo, 2020). For extraction, 4 mL of hexane was added to 100 mg of the sample, vortexed for 2 minutes, and centrifuged at 3000 rpm for 4 minutes. The hexane extract was transferred to a derivatization tube, evaporated under nitrogen, and treated with 1 mL of 2% NaOH at 90 °C for 5 minutes. After cooling, derivatization was performed by adding 1 ml of BF3 and heating for 30 minutes. Extraction with 2 mL of hexane was followed by GC-MS analysis (Griffiths *et al.* 2010).

The GC-MS analysis was performed using an Agilent 7890B Network GC System equipped with an Agilent 5977B MSD detector. Separation was achieved using an Agilent HP-5MS column (0.25 mm × 30 m × 0.25 µm). The inlet temperature was set to 290 °C, and the oven temperature was programmed as follows: 170 °C (1 minute), increased at 2 °C/min to 180 °C, then at 5 °C/min to 275 °C (5 minutes hold). The carrier gas flow rate was maintained at 1.0 mL/min (constant). The mass spectrometer conditions were set as follows: MS Quad temperature at 150 °C, MS Source temperature at 230 °C, and a scan range of 20 - 700 amu. A 1 µL sample was injected with a solvent delay of 0 minute. Each fatty acid was identified based on its Retention Time (RT) and mass spectral match with the Wiley 8.0 library. The RT values were recorded to facilitate compound identification and comparison across samples. Fatty acids were quantified using relative peak area normalization, and results were expressed as a percentage of total identified fatty acids to provide a normalized profile for each microalgal isolate (Patel *et al.* 2018). The relative abundance of each fatty acid was determined using the peak area normalization method, calculated as follows:

$$\% \text{ Normalization} = \left(\frac{\text{Peak Area of individual Fatty Acid}}{\text{Total Peak Area of All Fatty Acid}} \right) \text{X } 100$$

Data Analysis

The data obtained from the study were analyzed using an exploratory descriptive method to identify microalgae types from each sampling location. Morphological characteristics were examined under a light microscope, and identification was conducted using taxonomic keys. The fatty acid profile test was conducted on successfully cultivated microalgae cells using GC-MS. Peak identification was performed by comparing the mass spectra with reference spectra from the Wiley 8.0 library, and the relative abundance of each fatty acid was determined using peak area normalization. Data were displayed in tabular form and interpreted based on the sampling locations.

RESULTS AND DISCUSSION

Microalgae Isolates

Four distinct microalgae isolates were obtained from the ITS Dormitory Pond (Table 2). The isolation process involved serial dilution and the streak plate method, repeated until pure isolates were obtained, followed by periodic microscopic observations to confirm the purity of each isolate (Fernandez-Valenzuela *et al.* 2021).

Table 2 Microalgae isolate code

No.	Isolate Code	Sampling Location	Color
1.	HDB3a	Pond B (Station 3)	Green
2.	16HtDB2b	Pond B (Station 2)	Dark Green
3.	17HtDB2b	Pond B (Station 2)	Dark Green
4.	KD3a	Pond B (Station 3)	Yellow

Morphological Identification of Microalgae Isolates

The isolate HDB3a was identified as having round cells with diameters ranging from 4 µm to 10 µm. These cells formed small colonies of 4, 8, 16, or more cells, embedded in a transparent

mucilaginous matrix. Each cell contained one or more cup-shaped chloroplasts with chlorophyll, giving them a green appearance. Pyrenoids were present within the chloroplasts, playing a role in carbon storage and metabolism (Fig. 2, A1). The morphological characteristics of the isolate align with those described by Bellinger & Sigee (2015), particularly the spherical shape, mucilaginous colony formation, and cup-shaped chloroplasts, confirming its classification within the genus *Dictyosphaerium*. Krienitz & Bock (2010) also reported that *Dictyosphaerium* species typically form colonies as a defense mechanism against environmental stress. Additionally, Bock *et al.* (2011) found that *Dictyosphaerium*-like species develop colonies through mucilaginous stalks originating from remnants of the mother cell, suggesting possible polyphyly within the *Chlorella*ceae family. Mikhailyuk *et al.* (2020) further demonstrated the genus's morphological diversity by identifying *Dictyosphaerium*-like forms in terrestrial habitats. The presence of this isolate in a pond affected by domestic waste suggested its ability to thrive in nutrient-rich conditions. Its colony formation and chloroplast structures further support its adaptability to environmental changes.

Based on observations, the isolate coded 16HtDB2b had oval-shaped cells with diameters ranging from 10 to 17 µm. The transparent and smooth cell walls lacked any distinct ornamentation or structural modifications. A large, cup-shaped chloroplast occupied most of the cell's interior, containing chlorophyll pigments and pyrenoids, which functioned as storage centers for photosynthetic products, such as starch. These microalgae exhibited motility, suggesting the presence of flagella, although the flagella were not clearly observed (Fig. 2, B1). The morphological characteristics of this isolate align with the description of the genus *Vitreochlamys* provided by Wehr *et al.* (2015). *Vitreochlamys* is a unicellular green flagellate with a smooth, swollen cell wall that separates from the protoplast and features a prominent anterior papilla. Vegetative cells are solitary, biflagellate, and broadly spindle-shaped, pyriform, or ellipsoidal. They typically measure 24 - 34 µm in length and 10 - 22 µm in width, with flagella that are equal to or slightly shorter than the cell length. Two contractile vacuoles are positioned near the base of the flagella. The chloroplast has multiple lobes and contains 4 - 8 similarly sized pyrenoids, which are ellipsoidal, ovoid, or bean-shaped. These pyrenoids are surrounded by two large starch grains and are scattered throughout the chloroplast (Nakazawa *et al.* 2001).

Observations showed that the isolate coded 17HtDB2b had ellipsoid or cylindrical cells, measuring approximately 10 - 30 µm in length and 5 - 15 µm in width. The cell walls featured a complex structure with prominent spines or projections at both ends. The isolate formed colonies of 2 cells to 4 cells arranged in linear or flat formations. These microalgae were non-motile, as they lacked flagella (Fig. 2, C1). The morphological characteristics of this isolate are consistent with the genus *Desmodesmus*, as described by Bellinger & Sigee (2015). The spines in *Desmodesmus* serve as a defense mechanism against microzooplankton predation, as their rigid structure makes ingestion difficult (Trainor 1996). Additionally, the spines enhance buoyancy, helping the cells remain in the photic zone for optimal photosynthesis (Zhu *et al.* 2018). The surface morphology of *Desmodesmus* species, observed under SEM, varied significantly. For example, *D. armatus* exhibited a warty surface, *D. quadricauda* had a net-like structure, and *D. serratus* displayed a star-like pattern. Many *Desmodesmus* species show phenotypic plasticity, alternating between unicellular and colonial forms, as well as spiny and non-spiny variants. Environmental factors influence these transitions; for instance, *D. subspicatus* and *D. armatus* develop spiny colonies under low nutrient conditions, while unicellular forms dominate in phosphorus- or nitrogen-rich environments. When iron availability is low, both colonies and unicellular forms become non-spiny (Shubert *et al.* 2014).

The isolate coded KD3a consisted of small, round, single cells with diameters ranging from 2 µm to 10 µm. Each cell contained a large, parietal chloroplast occupying most of the cell volume, giving it a green color due to the presence of chlorophyll. The chloroplasts also contained pyrenoids, which facilitate photosynthesis. These microalgae were non-motile as they lacked flagella (Fig. 2, D1). The morphological characteristics of this isolate align with those of the genus *Chlorella*, as described by Bellinger & Sigee (2015). *Chlorella* species are unicellular green algae characterized by spherical or ovoid cells, typically measuring 2 - 10 µm in diameter. Their robust cell walls are composed of cellulose and pectin, providing structural integrity (Takeda 2004). The genus is

distinguished by a single, cup-shaped chloroplast with a prominent pyrenoid, essential for carbon fixation and starch storage. *Chlorella* is commonly found in nutrient-rich aquatic environments, but can be easily overlooked due to its small size. Its adaptability to various environmental conditions, including polluted waters, makes it a significant genus in both ecological and biotechnological studies (Bellinger & Sigee 2015).

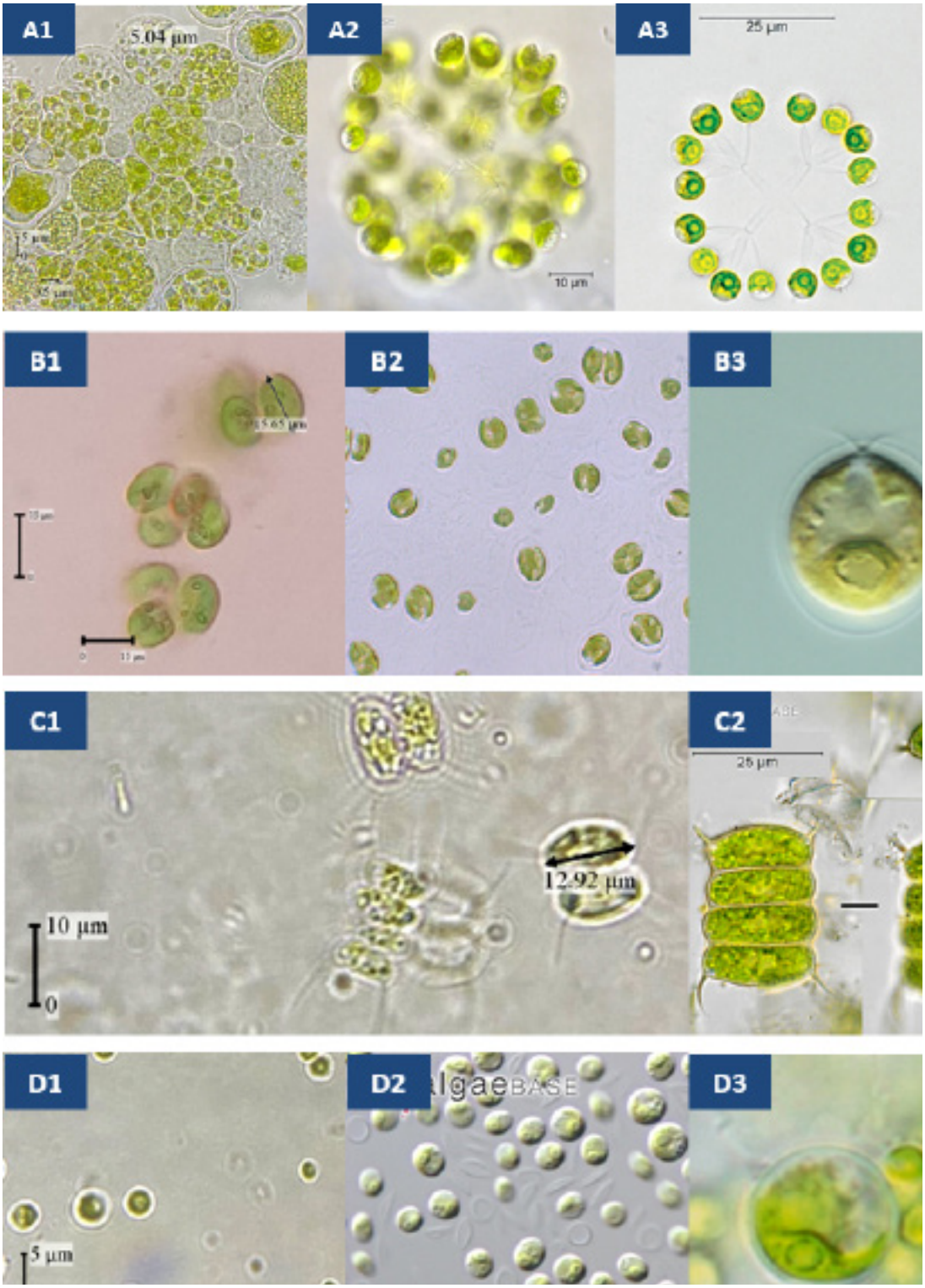


Figure 2 Observed microalgae in this study that have been identified by comparing to literatures

Notes: A1 = observed *Dictyosphaerium* (HDB3a); A2 = *Dictyosphaerium granulatum* (algaebase.org); A3 = *Dictyosphaerium granulatum* (Wehr *et al.* 2015);
B1 = observed *Vitreochlamys* (16HtDB2b); B2 = *Vitreochlamys incisa* (ccap.ac.uk); B3 = *Vitreochlamys aulata* (Wehr *et al.* 2015);
C1 = observed *Desmodesmus* (17HtDB2b); C2 = *Desmodesmus communis* (algaebase.org);
D1 = observed *Chlorella*; D2 = *Chlorella vulgaris* (algaebase.org); D3 = *Chlorella vulgaris* (Wehr *et al.* 2015).

Fatty Acid Profile Analysis

The harvesting of microalgal isolates was performed prior to conducting fatty acid profile analysis. The optimal harvest time was determined based on the growth curve of the microalgae, specifically during the early stationary phase. This phase is crucial as it marks the onset of metabolite accumulation, including lipids. During the early stationary phase, cell density reaches a constant and maximum level, resulting in the highest biomass concentration (Ferreira *et al.* 2019). The early stationary phase can be observed through the microalgae growth curve (Fig. 3). According to the growth curve, the harvest times for the microalgae were as follows: *Dictyosphaerium* was harvested on day 16, *Vitreochlamys* on day 15, and both *Desmodesmus* and *Chlorella* on day 13.

The study results indicated that the microalgal species with the highest total fatty acid content was *Dictyosphaerium* (30.18%), followed by *Desmodesmus* (25.72%), *Vitreochlamys* (21.02%), and *Chlorella* (4.59%). The fatty acid composition of microalgae isolates from the ITS Dormitory Pond Waters showed significant variation. The fatty acid profile test results indicated that most isolates had high fatty acid content, particularly elaidic acid (C18:1), palmitic acid (C16:0), and stearic acid (C18:0) (Table 3).

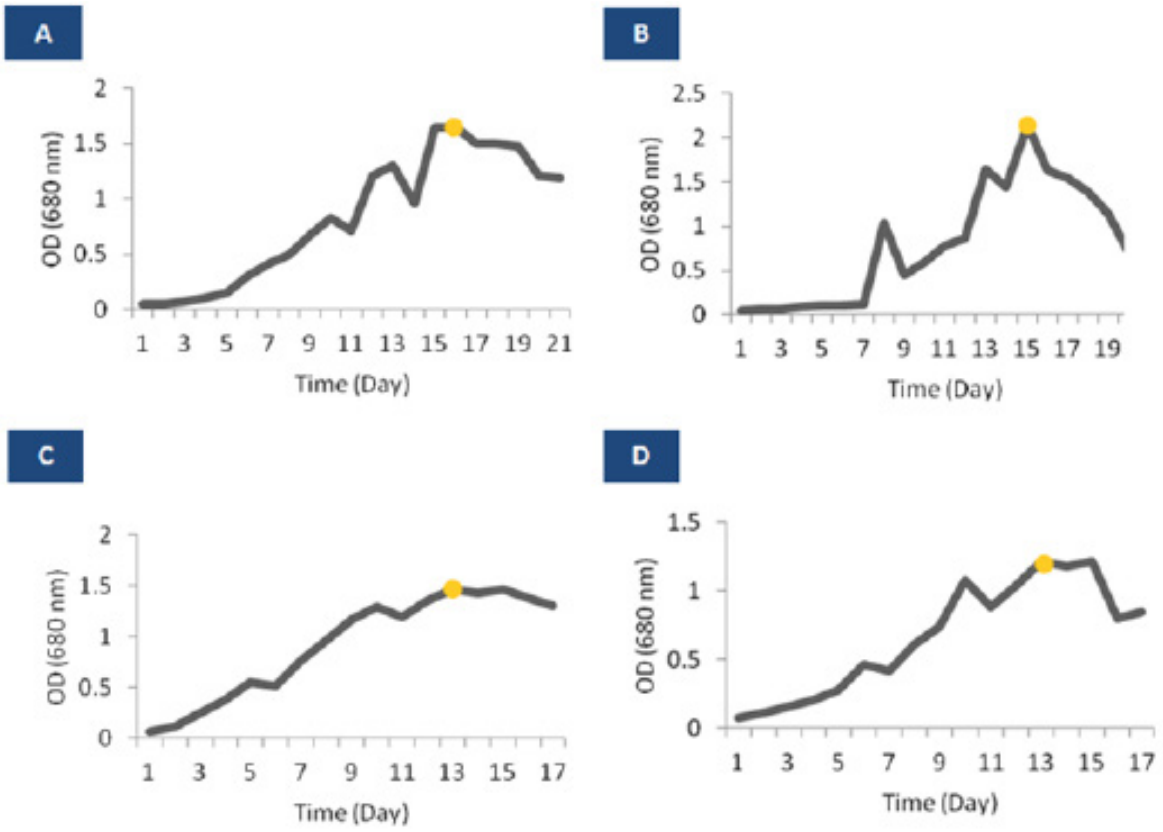


Figure 3 Growth curves of microalgal isolates based on optical density (OD) at 680 nm over time
Notes: A = *Dictyosphaerium*; B = *Vitreochlamys*; C = *Desmodesmus*; D = *Chlorella*; the yellow dots indicate the optimal harvest time.

Table 3 Fatty acid profile data of microalgal isolates from the ITS Dormitory Pond Waters

No.	Retention time	Component name	% Normalization			
			Dictyosphaerium	*Vitreochlamys*	*Desmodesmus*	*Chlorella*
1.	3.40	Lauric Aci (C12:0)	0.11	-	2.33	-
2.	6.04	Myristic Acid (C14:0)	0.85	0.52	1.41	0.01
3.	8.20	Myristic Acid (C14:0)	0.20	-	-	-
4.	8.17	Pentadecanoic Acid (C5:0)	-	-	0.26	-
5.	10.20	Hexadienoic Acid (C6:2)**	-	-	0.32	0.22
6.	10.57	Palmitic Acid (C16:0)	-	-	1.02	-
7.	11.40	Palmitic Acid (C16:0)	9.32	6.83	6.49	1.21
8.	14.52	Margaric Acid (C17:0)	-	-	0.16	-
9.	16.41	Linoleic Acid (C18:2)**	0.33	-	2.46	0.48
10.	16.62	α-Linoleic Acid (C18:3(ω-3))**	-	-	-	1.97
11.	16.70	Elaidic Acid (C18:1)*	12.28	10.19	7.39	-
12.	17.27	Stearic Acid (C18:0)	5.52	2.49	2.47	0.44
13.	20.17	Arachidonic Acid (C20:4(ω-6))**	-	-	0.44	-
14.	20.55	Eicosatrienoic Acid (C20:3(ω-3))**	-	-	0.22	-
15.	21.59	Arachidic Acid (C20:0)	0.54	0.35	0.05	0.26
16.	23.87	Eicosapentaenoic Acid (C20:5(ω-3))**	-	-	0.70	-
17.	25.20	Behenic Acid (C22:0)	1.03	0.64	-	-
Total SFA			17.57	10.83	14.19	1.92
Total MUFA			12.28	10.19	7.39	-
Total PUFA			0.33	-	4.14	2.67
Total Fatty Acids			**30.18**	**21.02**	**25.72**	**4.59**

Notes: * = monounsaturated fatty acids; ** = polyunsaturated fatty acids.

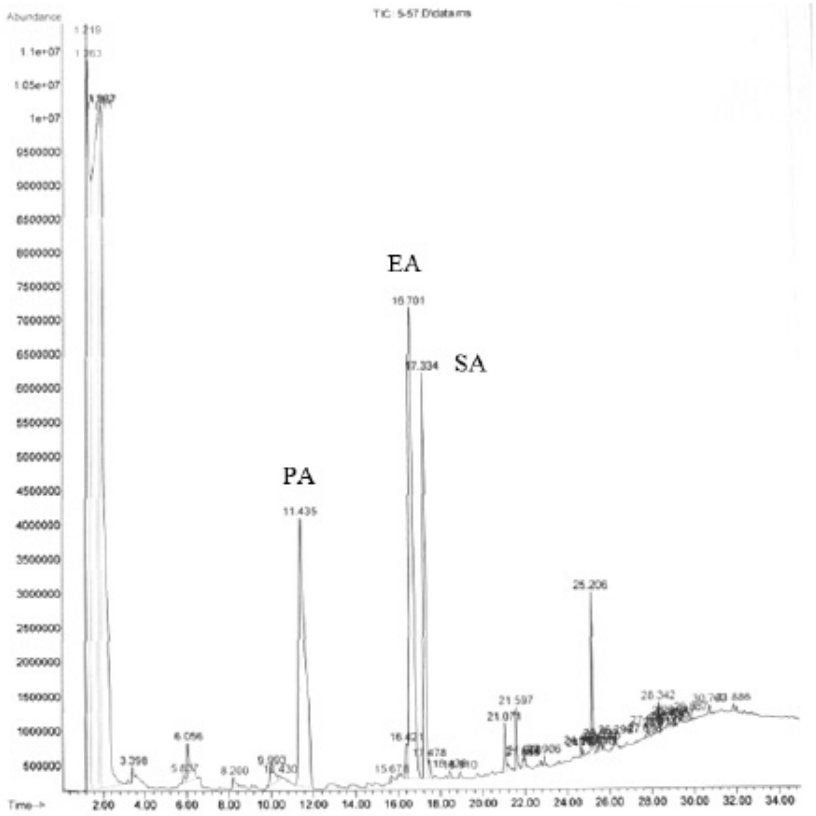
Dictyosphaerium exhibited the highest saturated fatty acid (SFA) content among the four microalgae species, with a total of 17.57% comprising lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, and behenic acid. The species also showed the highest level of monounsaturated fatty acids (MUFA), at 12.28%, predominantly as elaidic acid. Conversely, *Dictyosphaerium* had a lower content of polyunsaturated fatty acids (PUFA), amounting to 0.33%, primarily as linoleic acid (Fig. 4, A). In contrast, Nayana *et al.* (2018) reported that *Dictyosphaerium* contained the highest levels of SFA, specifically palmitic acid (22.64%). The MUFA accounted for 44.24%, including oleic acid (38.08%) and palmitoleic acid (6.16%). The PUFA content was 8.93%, with linoleic acid being the predominant PUFA. The discrepancy between the results of this study and those reported by Nayana *et al.* (2018) may be attributed to differences in cultivation conditions, which influence the composition of fatty acids produced during growth.

Vitreochlamys exhibited an SFA content of 10.83%, consisting of myristic acid, palmitic acid, stearic acid, arachidic acid, and behenic acid. The MUFA content was 10.19%, predominantly as elaidic acid, with no detected PUFA (Fig. 4, B). Research on the fatty acid profile of the genus *Vitreochlamys* is currently limited. Comparable studies within the family Chlamydomonadaceae can be observed, such as in *Chlamydomonas* sp., which contains SFA, including myristic acid (2.62%) and palmitic acid (78.35%). This species also has MUFA, like palmitoleic acid (4.26%) and oleic acid (2.48%), and PUFA, such as linoleic acid (3.49%) and arachidonic acid (3.18%). Palmitic acid (C16:0) is the most abundant fatty acid in *Chlamydomonas* sp., accounting for about 85% of the total fatty acid content, with 9% being PUFA (Morando-Grijalva *et al.* 2020).

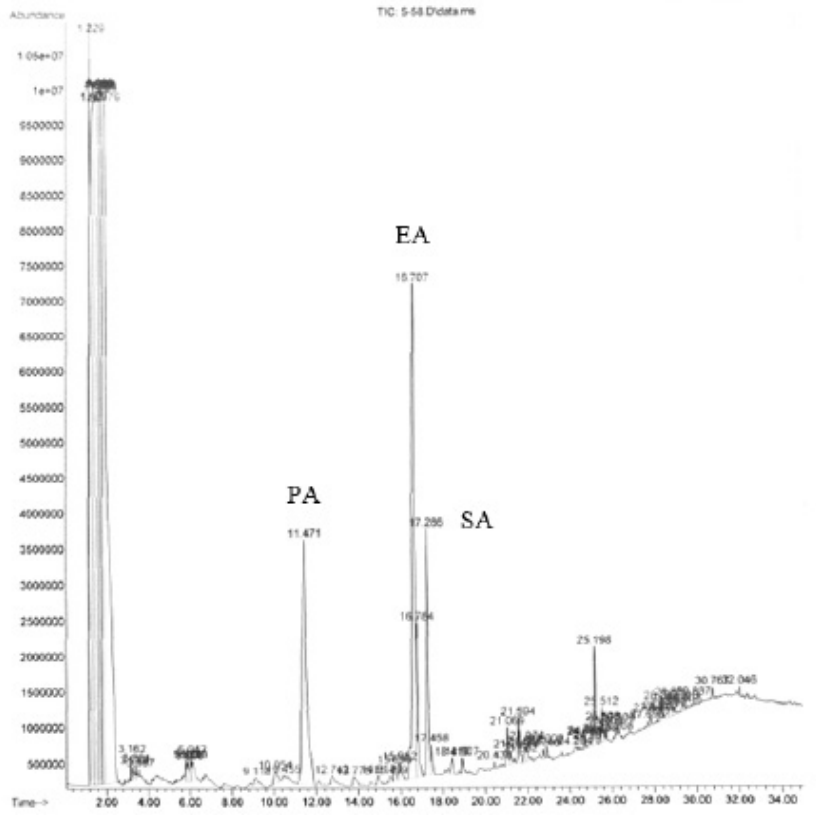
Desmodesmus exhibited an SFA content of 14.19%, which includes lauric acid, myristic acid, pentadecanoic acid, palmitic acid, margaric acid, stearic acid, and arachidic acid. The MUFA content was 7.39%, primarily as elaidic acid. Notably, *Desmodesmus* had the highest PUFA content among the four species analyzed, with a total of 4.14%, including hexadecanoic acid, linoleic acid, arachidonic acid, eicosatrienoic acid, and eicosapentaenoic acid (Fig. 4, C). In other studies, *Desmodesmus* was found to contain different compounds under autotrophic (Bourdeau *et al.* 2017), heterotrophic (Deschênes *et al.* 2015), and mixotrophic growth conditions (Okpozu *et al.* 2019).

Chlorella exhibited an SFA content of 1.92%, which included myristic acid, palmitic acid, stearic acid, and arachidic acid. The PUFA content was 2.67%, comprising hexadecanoic acid, linoleic acid, and α-linolenic acid. Notably, no MUFA were detected in *Chlorella* in this research (Fig. 4, D). According to Rushan *et al.* (2021), *Chlorella* cultivated in BG-11, JM, and BBM media displayed typical fatty acid compositions found in microalgal biomass, including palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). The highest SFA content was observed in *Chlorella* cultivated with BBM, followed by JM and BG-11 media. The highest SFA composition in BBM-cultivated *Chlorella* was attributed to C16:0 (40.74%) and C18:0 (33.33%) of total fatty acid methyl esters (FAME). The highest UFA composition was found in BBM due to the presence of C18:1 (11.11%), C18:2 (7.41%), and C18:3, (3.7%) of total FAME. This shows that using different media will produce different fatty acid contents (Rushan *et al.* 2021).

A


EA
SA
PA
11.435
16.701
17.334
25.206
Time-->
2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00

B


EA
SA
PA
11.471
16.707
17.286
25.198
Time-->
2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00

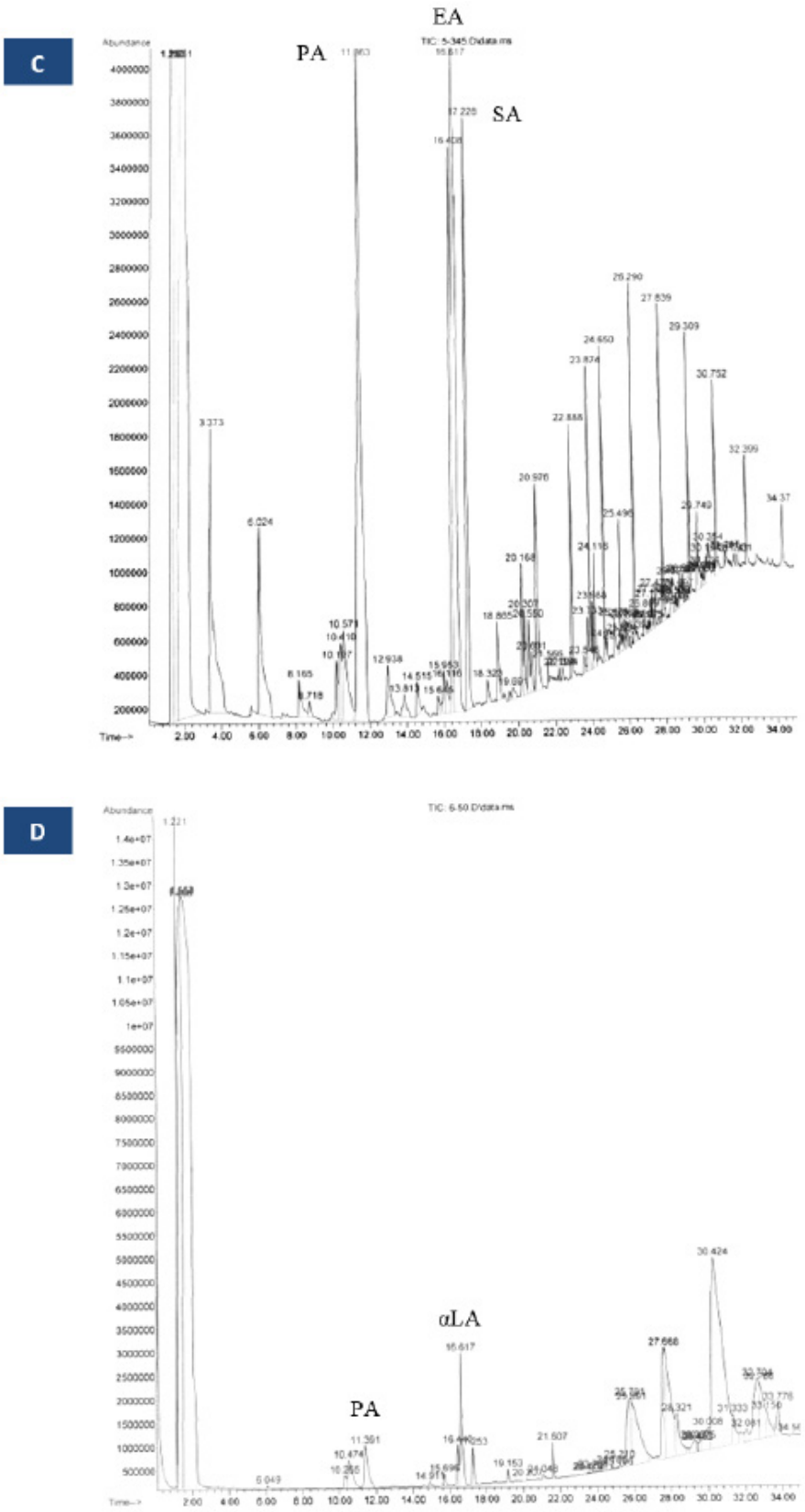


Figure 4 Chromatogram of fatty acid profile

Notes: A = *Dictyosphaerium*; B = *Vitreochlamys*; C = *Desmodesmus*; D *Chlorella*;
EA = elaidic acid; PA = palmitic acid; SA = stearic acid; αLA = α-linoleic acid.

CONCLUSION

This study successfully identified various microalgae from the waters of ITS Dormitory Pond in Surabaya, including *Dictyosphaerium*, *Vitreochlamys*, *Desmodesmus*, and *Chlorella*. The fatty acid profiles of the isolated microalgae species varied. *Dictyosphaerium* was found to have a saturated fatty acid (SFA) content of 17.57%, monounsaturated fatty acid (MUFA) content of 12.28%, and polyunsaturated fatty acid (PUFA) content of 0.33%. *Vitreochlamys* exhibited an SFA content of 10.83% and a MUFA content of 10.19%, with no detected PUFA. *Desmodesmus* showed an SFA content of 14.19%, MUFA content of 7.39%, and PUFA content of 4.14%. *Chlorella* had an SFA content of 1.92% and a PUFA content of 2.67%, with no detected MUFA. Future research should focus on optimizing culture conditions to enhance lipid accumulation and improve fatty acid composition for commercial applications. Investigating genetic and metabolic pathways could further increase lipid productivity and tailor fatty acid profiles for specific industrial uses. Additionally, evaluating large-scale cultivation feasibility and sustainability aspects will be essential for developing microalgae-based bioresources in biofuels, nutraceuticals, and pharmaceuticals.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to Ratna Syifa'a Rahmahana and Etika Ziadana Al Husna for their invaluable contributions as laboratory partners, particularly in data collection, analysis, and interpretation for this project. The authors also extend their appreciation to the Sepuluh Nopember Institute of Technology for its support and the provision of research facilities. This study was funded by the ITS Scientific Research Scheme 2023.

REFERENCES

Abdelkarim OH, Wijffels RH, Barbosa MJ. 2024. Microalgal lipid production: A comparative analysis of *Nannochloropsis* and *Microchloropsis* strains. J Appl Phycol 37(1):15-34.

Ardiansyah SR, Orlando AM, Rahman A, Prihantini NB, Nasruddin. 2018. Effect of aeration in simple photobioreactor system for biomass production of *Synechococcus* sp. (Cyanobacteria) HS-7 and HS-9 as biofuel feedstock. E3S Web Conf 67(02006):1-6.

Bellinger EG, Sigee DC. 2015. Freshwater algae: Identification, enumeration and use as bioindicators. New Delhi (IN): John Wiley & Sons, Ltd.

Bock C, Pröschold T, Krienitz L. 2011. Updating the genus *Dictyosphaerium* and description of *Mucidosphaerium* gen. nov. (Trebouxiophyceae) based on morphological and molecular data. J Phycol 47(3):638-52.

Bourdeau N, Bélanger-Lépine F, Adjallé K, Dubois-Caléro N, Dosnon-Olette R, Samson G, Barnabé S. 2017. Mixotrophic cultivation of an algae-bacteria consortium in aluminium smelter wastewaters (Quebec, Canada): High nitrogen concentration increases overall lipid production. Ind Biotech 13(5):260-9.

Chaidir Z, Fadjria N, Armaini, Zainul R. 2016. Isolation and molecular identification of freshwater microalgae in Maninjau Lake West Sumatra. Sch Res Lib 8(20):177-87.

Chen W, Li T, Du S, Chen H, Wang Q. 2023. Microalgal polyunsaturated fatty acids: Hotspots and production techniques. Front Bioeng Biotechnol 11(1146881):1-22.

Chen YC, Yeh KL, Aisyah R, Lee DJ, Chang JS. 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. Bioresour Technol 102:71-81.

Chiu H, Kuo C. 2020. Gas chromatography-mass spectrometry-based analytical strategies for fatty acid analysis in biological samples. J Food Drug Anal 28(1):60-73.

Deschênes J, Boudreau A, Tremblay R. 2015. Mixotrophic production of microalgae in pilot-scale photobioreactors: Practicability and process considerations. Algal Res 10:80-6.

Dolganyuk V, Belova D, Babich O, Prosekov A, Ivanova S, Katserov D, ..., Sukhikh S. 2020. Microalgae: A promising source of valuable bioproducts. Biomolecules 10(8):1153.

Elisabeth B, Rayen F, Behnam T, 2021. Microalgae culture quality indicators: A review. Crit Rev Biotechnol 41(4):451-73.

Fernandez-Vanenzuela S, Ruvalcaba FC, Beltran-Rocha JC, Claudio PMS. 2021. Isolation and culturing axenic microalgae: Mini–Review. Open Microbiol J 15(1):111-19.

Ferreira GF, Pinto LFR, Carvalho PO, Coelho MB, Eberlin MN, Filho RM, Fregolente LV. 2019. Biomass and lipid characterization of microalgae genera *Botryococcus, Chlorella*, and *Desmodesmus* aiming high-value fatty acid production. Biomass Convers Biorefin 11:1675-89.

Griffiths MJ, Van Hille RP, Harrison STL. 2010. Selection of direct transesterification as the preferred method for assay of fatty acid content of microalgae. Lipids 45:1053-60.

Kawaroe M, Prartono T, Rachmat A, Sari DW, Augustine D. 2010. Mikroalga: Potensi dan pemanfaatannya untuk produksi bio bahan bakar [Microalgae: Potential and use for biofuel production]. Bogor (ID): IPB Press.

Krienitz L, Bock C. 2010. Polyphiletic origin of the *Dictyosphaerium* Morphotype within *Chlorella*ceae (Trebouxiophyceae). J Phycol 46(3):559-63.

Lee K, Eisterhold ML, Rindi F, Palanisami S, Nam PK. 2014. Isolation and screening of microalgae from natural habitats in the midwestern United States of America for biomass and biodiesel sources. J Nat Sci Biol Med 5(2):333-39.

Mazidah R, Mulyadi A, Nasution S. 2013. Tingkat Pencemaran Perairan Danau Buatan Pekanbaru Ditinjau dari Parameter Fisika, Kimia, dan Biologi [Pekanbaru Artificial Lake Water Pollution Level Seen from Physical, Chemical and Biological Parameters]. Riau (ID): Pusat Penelitian Lingkungan Hidup.

Mikhailyuk T, Holzinger A, Tsarenko P, Glaser K. 2020. *Dictyosphaerium*-like morphotype in terrestrial algae: What is Xero*chlorella* (Trebouxiophyceae, Chlorophyta)?. J Phycol 56(3):1-16.

Milano J, Ong HW, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V. 2016. Microalgae biofuels as an alternative to fossil fuel for power generation. Renew Sustain Energy Rev 58:180-97.

Morando-Grijalva CA, Vázquez-Larios AL, Alcántara-Hernández RJ, Ortega-Clemente LA, Robledo-Narváez PN. 2020. Isolation of a freshwater microalgae and its application for the treatment of wastewater and obtaining fatty acids from tilapia cultivation. Environ Sci Pollut Res 27(23):28575-584.

Nayana B, Venkatesh HN, Sudharsana TN, Manjunath K, Mohana DC. 2018. Isolation and utilization of *Dictyosphaerium ehrenbergianum* Nageli for biodiesel production: Lipid extraction and biodiesel property analysis. Biofuels 11:885-92.

Okpozu OO, Ogbonna IO, Ikwebe J, Ogbonna JC. 2019. Phycoremediation of cassava wastewater by *Desmodesmus armatus* and the concomitant accumulation of lipids for biodiesel production. Bioresour Technol Rep 7(100255):1-7.

Patel MK, Das S, Thakur JK. 2018. GC-MS-based analysis of methanol: Chloroform-extracted fatty acids from plant tissues. Bio Protoc 8(18):1-9.

Rushan NH, Mat Yasin NH, Said FM. 2021. The effect of culture medium on the oil yield and fatty acid methyl ester of freshwater microalgae *Chlorella vulgaris*. Chem Eng Comms 208(4):592-600.

Saputro TB, Purwani KI, Ermavitalini D, Saifulloh AF. 2019. Isolation of high lipid content microalgae from Wonorejo River, Surabaya, Indonesia and its identification using *rbcL* marker gene. Biodiversitas 20(5):1380-88.

Shang X, Yang Y, Zan Y, Sun Z, Lu Z, Sun J. 2024. Effects of temperature on the growth, total lipid content and fatty acid composition of *Skeletonema dohrnii*. Front Mar Sci 1-9.

Shubert E, Wilk-Wozniak E, Ligeza S. 2014. An autecological investigation of *Desmodesmus*: implications for ecology and taxonomy. Plant Ecol Evol 147(2):202-12.

Takeda H. 2004. Sugar composion of cell wall and the taxonomy of *Chlorella* (Chlorophyceae). J Phycol 27(2):224-32.

Trainor FR. 1996. Reproduction in *Scenedesmus*. Algae 11(2):183-201.

Wehr JD, Sheath RG, Kociolek JP. 2015. Freshwater algae of North America. San Diego (US): Academic Press.

Wu J, Gu X, Yang D, Xu S, Wang S, Chen X, Wang Z. 2021. Bioactive substances and potentiality of marine microalgae. Food Sci Nutr 9(9):5279-92.

Zhu W, Li Y, Fei X, Deng X. 2018. Nutrient removal and algal community variation from urban river with the isolated microalgal strains *Chlorella* sp. and *Scenedesmus* sp. J Water Resour Prot 10(9):884-95.