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COMPARATIVE LIPIDOMICS OF FRESHWATER MICROALGAE REVEALS BIODIESEL POTENTIAL OF CHLAMYDOMONAS REINHARDTII, CHLORELLA VULGARIS, AND SPIRULINA PLATENSIS FROM THANE DISTRICT (MS)

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Abstract
The microalgae have emerged as viable renewable feedstocks for sustainable biodiesel production due to their quick growth rates, high lipid productivity, and flexibility to a wide range of environmental conditions. The current study focus on the lipidomic profiles and biodiesel potential of three freshwater microalgae, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Spirulina platensis*, obtained from freshwater environments in thane District, Maharashtra, India. Algal biomass was grown in a controlled laboratory environment (BG-11 medium) and lipid extracted using the Bligh and Dyer technique. Lipid studies were done quantitatively and qualitatively using HPLC and Gas Chromatography-Mass Spectrometry (GC-MS). *Chlorella vulgaris* had the greatest lipid content of the examined species (35%), followed by *Chlamydomonas reinhardtii* (22%), and *Spirulina platensis* (12%). GC-MS analysis confirmed the presence of palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2), all of which are regarded beneficial fatty acids for biodiesel generation. The conversion efficiency of biodiesel was calculated to be 89%, 74%, and 58% for *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Spirulina platensis* respectively. The data show that *Chlorella vulgaris* has the best biodiesel feedstock properties among the examined species, highlighting the bioenergy potential of indigenous freshwater algae from Thane district.

Keywords: Biodiesel, Lipidomics, Microalgae, GC–MS, HPLC, Fatty Acid Methyl Ester, Biofuel, Thane

Introduction

The global energy demands and environmental concerns about fossil fuel usage have fuelled the hunt for renewable and sustainable energy sources [Abomohra, A. E *et.al*., 2020]. Biodiesel made from biological feedstocks is a potential option because to its biodegradability, low toxicity, and lower greenhouse gas emissions [Ahmad, A. *et al*., 2011]. The microalgae have

gained popularity as a feedstock due to their high photosynthetic efficiency, fast biomass build-up, and high lipid content [Chisti, Y. 2007 & Converti, A *et al* 2009]

The freshwater microalgae are especially important since they are abundant and adaptable to local environmental circumstances [Chandanshive V R & Cholke P B., 2021]. *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Spirulina platensis* are renowned for their unique biochemical makeup and possible use in biofuel generation (Plate No.1; Image No.1, 2 &3). Lipid profiling using modern analytical methods like HPLC and GC-MS allows for the detection of fatty acid content and biodiesel applicability [Demirbas, A & Demirbas, M. F. 2011].

The Thane District of Maharashtra, has various freshwater environments that sustain abundant algal variety. There is little information available about the biodiesel potential of indigenous algae strains from this region. The current study aims to (i) compare lipid accumulation in various freshwater microalgae, (ii) describe their lipid composition using HPLC and GC-MS, and (iii) assess their appropriateness as biodiesel feedstocks.

Materials and methods

Study Area

Freshwater samples were collected from ponds, reservoirs, and streams in Thane District, Maharashtra, India (**19.1970° N and 72.9635°**).

Sample Collection and Isolation (Plate No.1; Image No. 4)

Water samples were collected in sterile containers and transported to the laboratory. Microalgae were isolated using serial dilution and cultured in BG-11 medium under controlled laboratory conditions.

Biomass Cultivation [Tamara Vujovic *et al*., 2025]

Pure cultures of *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Spirulina platensis* were maintained at,

Temperature: 25 ± 2°C, Photoperiod: 16:8 h (light: dark), Light intensity: 2000 lux, Culture duration: 21 days.

Lipid Extraction [Bligh, E. G., & Dyer, W. J. (1959)]

Total lipids were extracted using the Bligh and Dyer solvent extraction method employing chloroform: methanol (2:1 v/v). Lipid content was determined gravimetrically and expressed as percentage dry weight.

HPLC Analysis [Zhenming Zhong *et al*., 2009 & Hiroko Shibata *et al*., 2013]

Extracted lipids were analysed using reverse-phase HPLC equipped with a C18 column and UV detector. Lipid fractions including triglycerides, phospholipids, and glycolipids were quantified.

GC–MS Analysis [Huai-Hsuan Chiu & Ching-Hua Kuo 2019]

Fatty Acid Methyl Esters (FAMEs) were prepared through transesterification and analyzed using GC–MS. [HPLC and GC-MS were done in Aurangabad institute using standard references]

Results

Chlorella vulgaris has the greatest lipid content (35%) of all the examined species, followed by *Chlamydomonas reinhardtii* (22%) and *Spirulina platensis* (12%). According to these findings, *Chlorella vulgaris* is the most promising option for lipid-based processes like the manufacture of biodiesel. [Table No. 1]

Table No.1. Lipid Content (%)

Species	**Lipid Content (%)**
Chlorella vulgaris	**35**
Chlamydomonas reinhardtii	**22**
Spirulina platensis	**12**

The high oleic acid concentration of *Chlorella vulgaris* makes it stand out because it provides a good balance between oxidative stability and cold-flow characteristics, which is greatly desired for the synthesis of biodiesel. All species have high levels of saturated fatty acids (Palmitic + Stearic), which range from 37 to 38%. This suggests that fuel stability is typically good. *Spirulina platensis*, may be less appropriate for biodiesel because of its lower amounts of C18 fatty acids, but it could be useful in nutritional applications where fatty acid variety is advantageous. [Table No. 2]

Table 2. Major Fatty Acids Identified by GC–MS

Fatty Acid	***Chlorella* (%)**	***Chlamydomonas* (%)**	***Spirulina* (%)**
Palmitic Acid (C16:0)	28	25	22
Oleic Acid (C18:1)	34	26	18
Linoleic Acid (C18:2)	20	22	15
Stearic Acid (C18:0)	10	12	16

Chlorella vulgaris had the greatest biodiesel output (89%) of all the investigated species, followed by *Chlamydomonas reinhardtii* (74%). The *Spirulina platensis* (58%) had the lowest yield. These results show that, among the microalgae under study, *Chlorella vulgaris* is the most promising feedstock for biodiesel synthesis under the specified experimental circumstances. [Table No. 3]

Table 3. Estimated Biodiesel Conversion Yield

Species	Biodiesel Yield (%)
Chlorella vulgaris	89
Chlamydomonas reinhardtii	74
Spirulina platensis	58

Discussion

The study demonstrated significant variation in lipid accumulation among the investigated microalgae. *Chlorella vulgaris* exhibited the highest lipid content and biodiesel conversion efficiency, consistent with previous studies identifying this species as a promising biofuel feedstock [Griffiths,M. J., & Harrison S. T. L.2009]. The predominance of oleic and palmitic acids is advantageous for biodiesel quality due to improved cetane number, oxidative stability, and fuel performance [Hu, Q *et al.,* 2008][Plate No 2 : Image No. 1 &2].

GC–MS analysis confirmed that all three species contain substantial quantities of saturated and monounsaturated fatty acids [Kula M *et al*., 2016]. The comparatively lower lipid accumulation observed in *Spirulina platensis* may limit its suitability for large-scale biodiesel production despite its rapid biomass growth. HPLC chromatograms further indicated a higher proportion of neutral lipids in *Chlorella vulgaris*, which contributes directly to biodiesel yield [Plate No 2: Image No. 3 & 4].

Conclusion

Comparative lipidomic analysis revealed substantial differences in biodiesel potential among the studied freshwater microalgae. *Chlorella vulgaris* exhibited the highest lipid content, favorable fatty acid composition, and maximum biodiesel conversion efficiency, making it the most promising candidate for biofuel production. *Chlamydomonas reinhardtii* demonstrated moderate potential, whereas *Spirulina platensis* showed relatively lower lipid productivity [Li, Y *et al*., 2008]. The results highlight the significance of indigenous freshwater algal resources from Western India as sustainable feedstocks for future biodiesel development [Shibata, H *et al*., 2013]

Plate No.1

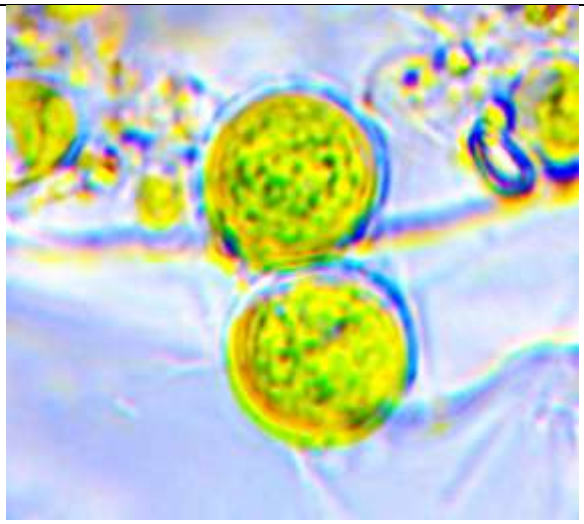


Image No.1. *Chlorella vulgaris*

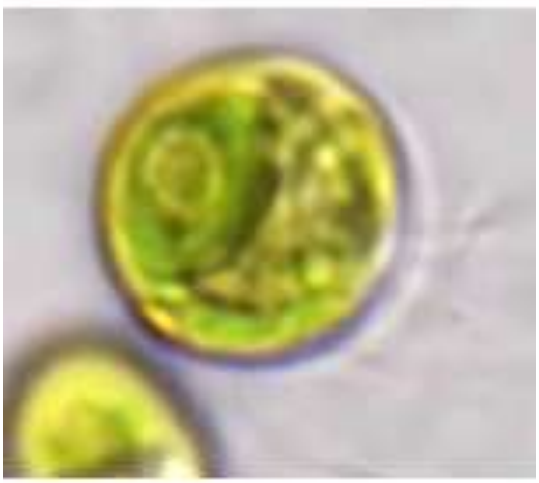


Image No. 2. *Chlamydomonas reinhardtii*

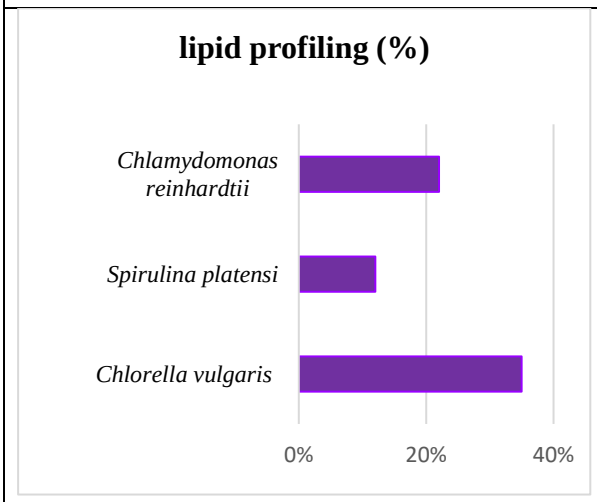


Image No.3. *Spirulina platensis*

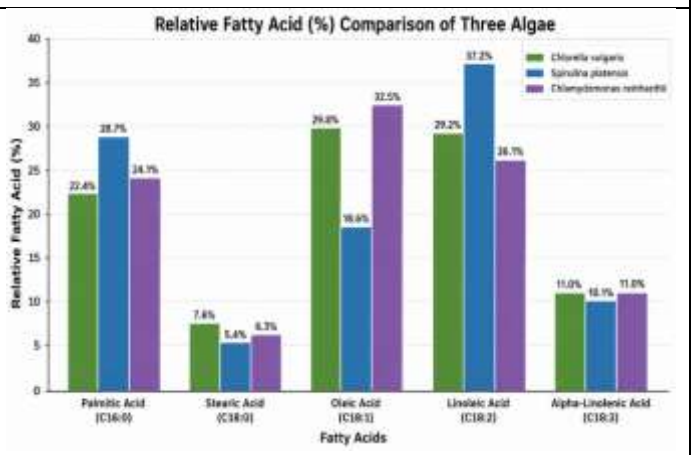


Image No.4. Culture of *Spirulina* (BG11)

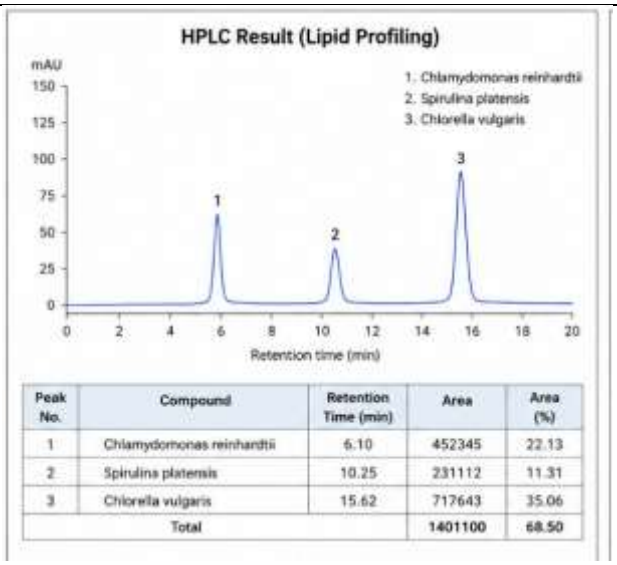
Plate No.2



Graph No.1

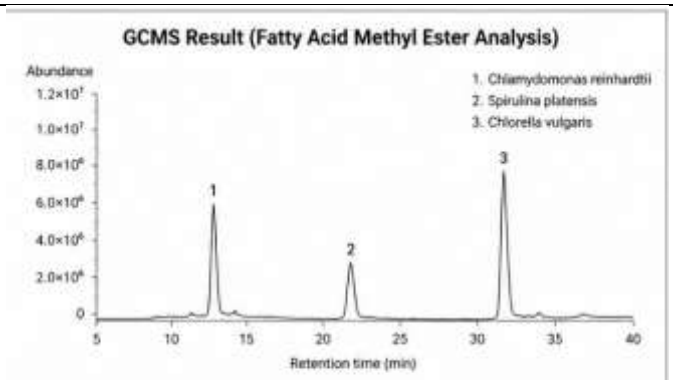


Graph No.2



Peak No.	Compound	Retention Time (min)	Area	Area (%)
1	Chlamydomonas reinhardtii	6.10	452345	22.13
2	Spirulina platensis	10.25	231112	11.31
3	Chlorella vulgaris	15.62	717643	35.06
Total			1401100	68.50

Graph No.3



Peak No.	Compound	Retention Time (min)	Area	Area (%)	Major Fatty Acids (Identified)
1	Chlamydomonas reinhardtii	14.32	5.18×10^{6}	22.05	C16:0, C18:1, C18:2
2	Spirulina platensis	21.45	2.64×10^{6}	11.24	C16:0, C18:1, C18:3
3	Chlorella vulgaris	32.18	8.26×10^{6}	35.20	C16:0, C18:1, C18:2
Total			1.61×10^{7}	68.49	

Graph No.4

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