First Report on Tertiary Amine as a Co2 Switchable Solvent for Hypersaline Trebouxiophycean Microalgae Towards Greener and Competent Lipid Extraction

Susaimanickam Anto  
National Institute of Technology Tiruchirappalli

M Premalatha  
National Institute of Technology Tiruchirappalli

Thangavel Mathimani (✉️ mathi.search@gmail.com)  
National Institute of Technology Tiruchirappalli

Research Article

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Abstract

Considering the momentous cost drivers in energy efficient algal biorefinery processes, a green alternative in the lipid extraction process from microalgae is anticipated. Switchable solvent system using tertiary amines namely DMBA (Dimethylbenzylamine), DMCHA (Dimethylcyclohexylamine), and DIPEA (Diisopropylethylamine) for lipid extraction from wet hypersaline microalgae was investigated in this study. Interestingly, showed that at 1:1 (v/v of fresh DMBA solvent: microalgal biomass), and for 1 h extraction time, the lipid yield was 41.9, 26.6, and 33.3% for *Chlorella* sp. NITT 05, *Chlorella* sp. NITT 02, and *Picochlorum* sp. NITT 04 respectively and for recovered DMBA solvent at 1:1 (v/v) and for 1 hour extraction time, the lipid yield was 40.8, 25.97, and 32%, respectively. Similarly, lipid extraction using DMCHA solvent for *Chlorella* sp. NITT 05, *Chlorella* sp. NITT 02, and *Picochlorum* sp. NITT 04 at 1:1 (v/v of solvent: microalgal biomass) and 1 h extraction time showed 34.28, 24.24 and 23.33% lipids, respectively for fresh solvent and 34.01, 24.24 and 23.18% for recovered solvent respectively; while DIPEA was not competent in lipid extraction from three tested microalgae. FAME profile shows the presence of major saturated fatty acid as C16:0 (~ 30%) and major unsaturated fatty acid as C18:1 (~17%).

1. Introduction

In the pursuit of gratifying the mainstream of energy mandate, fossil fuel takes the step front always. However, uncertainties in fossil fuel availability and its augmented greenhouse gases emission exemplify two major disputes viz., steady escalation in fuel price and climatic hitches like global warming\(^1\),\(^2\). Petroleum or fossil fuels are considered as a depleting energy reserve against growing demand due to their non-renewable feature, and unarguably they pose a potential threat to the transportation sector\(^3\). In this scenario, biodiesel is clearly emerging as an alternative supplement, which can be readily, introduced into the existing transportation infrastructure without engine modification\(^4\). Though, many feedstocks are being used for biodiesel, microalgae hold great promise as a hopeful production platform for biodiesel\(^5\) and carbon dioxide sequestration due their traits like less land requirement, high biomass and lipid productivity\(^2\),\(^6\), accumulation of non-polar glycerolipids (triacylglycerol)\(^7\), versatility to grow in fresh and seawater. However, microalgal biodiesel production still needs to throw light on resilient strain selection, pertinent lipid extraction method, and inexpensive harvesting technique. The key challenge that needs to be addressed with an emphasis on commercially viable microalgal biodiesel production is to ascertain an opposite, eco-friendly lipid extraction technique\(^8\). Till date, the supreme barrier for the development of green chemical-based extraction is that specific solvents are required to solvate specific lipid component type since each solvent has its own solubility. Reuse of solvent recovered through certain thermal processes (rotary evaporation or distillation) is also essential to obviate energy efficiency, but by doing so, would eventually escalate the energy and cost. Therefore, energy efficient and ecofriendly lipid extraction method is need of the hour as the present toxic solvent system with unproductive extraction efficiency cannot be an ideal choice. In a perspective of ecofriendly and reusability attributes, CO\(_2\) switchable solvent systems for lipid extraction would indeed be a great choice. CO\(_2\) switchable solvent system includes amine which can be primary, secondary, and tertiary amines. Among these, primary and
secondary amines were explored for algal lipid extraction in the recent years. In case of tertiary amines, DMCHA has been used for few microalgal lipid extractions. To best of our knowledge, this is the first study on using tertiary amine as a CO₂ switchable solvent for hypersaline microalgal strains as a greener and safe lipid extraction processes. Hence, in the present study tertiary amines such as DMBA, and DIPEA are used for lipid extraction from marine microalgal species with a comparison of DMCHA solvent. The aim of this study is to investigate the use of tertiary amines as a safer alternative for wet extraction of *Chlorella* sp. NITT 02, *Chlorella* sp. NITT 05, and *Picochlorum* sp. NITT 04. Lipid extracted from fresh solvent, the percentage of solvent recovery and the lipid extracted from the recycled solvents are comparatively studied. Further, fatty acid composition of lipids extracted using SPS system was analysed.

2. Results And Discussion

2.1. Habitat of microalgae

The microalgal sample isolated from the stagnant seawater of Kanyakumari district, Tamil Nadu (Latitude: 8° 7'28.10"N; Longitude: 77°29'16.89"E) was identified as *Picochlorum* sp. [Strain name: *Picochlorum* sp. NITT 04], which was characterized by tiny, unicellular, non-flagellated microalgae about ~2 µm in diameter (Table 1). The microalgal sample collected from the saltpan of Kanyakumari, Tamil Nadu (Latitude: 8° 6'38.91"N; Longitude: 77°29'13.43"E) was morphologically identified as *Chlorella* sp. [Strain name: *Chlorella* sp. NITT 02] characterized by unicellular, non-motile, round shaped microalgae. The other sample isolated from stagnant seawater in Muttukadu, Chennai (Latitude: 12° 48’ 45. 76” N; Longitude: 80° 14’ 37. 67” E) was morphologically identified as *Chlorella* sp. [Strain name: *Chlorella* sp. NITT 05]. Identified microalgal strains were initially grown in 50 mL conical flasks containing ASN-III medium and the temperature was maintained at 25°C ± 2°C with a light intensity of 1500 lux for 16:8 h light: dark photoperiod. All the primary stock cultures of isolated marine microalgae are maintained under the above-mentioned culture conditions and scaled up further to generate high voluminous biomass for subsequent studies.

| Isolate No. | Strain name            | Habitat              | Collection site             | GPS             |
|------------|------------------------|----------------------|----------------------------|-----------------|
| Isolate – 2| *Chlorella* sp. NITT 02| Saltpan              | Kanyakumari, Tamil Nadu    | 8° 6’38.91”N    |
| Isolate – 4| *Picochlorum* NITT 04  | Stagnant seawater    | Kanyakumari, Tamil Nadu    | 8° 7’28.10”N    |
| Isolate – 5| *Chlorella* sp. NITT 05| Stagnant seawater    | Muttukaddu, Chennai         | 12° 48’ 45. 76” N |

Table 1: Marine microalgal isolates and their geographical sites in South East Coast, India
2.2. Asynchronous growth metrics of microalgal strains

Fixed inoculum density of *Picochlorum* sp. NITT 04, *Chlorella* sp. NITT 02, and *Chlorella* sp. NITT 05 was analyzed for time course asynchronous growth kinetics from day 0 to 27 using triplicate experiments (Fig. 1). Of the strains tested, *Picochlorum* sp. NITT 04 was found to show higher cell density at about 2.23 OD on 27th day (Fig. 1c). The growth of *Picochlorum* sp. NITT 04 was gradually increased from day 0 till day 27; however after 27th day of its growth, no remarkable trend in growth rise was noticed. Similar to the results reported from this study, the OD value of *Picochlorum oklahomensis* after 25th day was around 2 and subsequently decreased over time. In the case of *Chlorella* strains, the initial OD of the *Chlorella* sp. NITT 02 and NITT 05 culture was 0.61 on day 0 and the OD got increased during the growth and the high culture density (maximal OD) was seen on cultivation day 27 which is 1.45 and 1.62 OD, respectively (Fig. 1a, 1b). The observation in *Chlorella* sp. NITT 02 & NITT 05, agrees to some extent with the study conducted by Chioccioli et al., (2014) demonstrating that *Chlorella vulgaris* reached stationary phase at around OD_{750} value of 1.3 when grown in TAP medium. Also, various recent reports in the growth pattern of different *Chlorella* sp. were in accordance with the results obtained from this study. The cell density obtained in this study was higher since the culture was grown in ambient condition without CO₂ purging. It could rise further by cultivating it in high strength medium purged with CO₂.

2.3. Lipid extraction by Switchable solvent system

In the present study, growth metrics of *Picochlorum* sp. NITT 04, *Chlorella* sp. NITT 02, and *Chlorella* sp. NITT 05 disclosed that higher biomass was produced on 27th of cultivation, but, no information on lipid content and yield has been retrieved, which is considered to be a important parameter for biodiesel production. Therefore, it is imperative to study lipid content of the strain to evaluate its feasibility for biodiesel and the lipid extraction was done using the biomass harvested on 27th day. Switchability nature of tertiary amine during lipid extraction from marine microalgae is illustrated in Fig. 3. The amount of lipid extracted by the solvent (fresh tertiary amine) indicated as “fresh” and the amount of lipid extracted by the solvent after first use, indicated as “recovered” in Fig. 4a were calculated gravimetrically. It is apparent that DMBA solvent was able to extract more lipids than DMCHA and DIPEA solvents. Also, *Chlorella* sp. NITT05 yielded significantly more lipids followed by the other two species. It is to be noted that, higher lipid at about 42% was extracted by both fresh and recovered solvent of DMBA from *Chlorella* sp. NITT05 whereas it was 23% using DMCHA. The other strain *Chlorella* sp. NITT02 yielded 27% with the use of fresh and recovered DMBA solvent. In the case of *Picochlororum* strain, higher lipid content of 33% was obtained from DMBA and 23% was obtained from DMCHA. On a similar note, lipids extracted from freeze dried *Botryococcus braunii* by DMCHA accounted to 22% of crude lipid yield. Another study by Samori et al., (2013) reported that the total lipid content of *D.communis, N.gaditana*, and *T.suecica* by DMCHA extraction was 29.2%, 57.9%, and 31.9% respectively. Among the tertiary amines used, the DIPEA was found to be inefficient in extracting lipids from all the strains and lipids extracted by DIPEA solvent was very less compared to other two solvents and restricted to switchability by forming salts after CO₂.
purging. This agrees with the existing literatures, where the formation of solid carbamate salts hinders
the reversion process by taking great time and requiring high temperature for conversion. Hence, the
choice is over liquid amines resulting in liquid carbamate salts allowing the ease of switchable nature
from liquid carbamate salts which forms the basis of SPS \(^{19,20}\). The lipids extracted by DIPEA were 10,
12.12 and 13.3% for Picochlorum sp. NITT 04, Chlorella sp. NITT 02, and Chlorella sp. NITT 05,
respectively. Hence from the observations, lipids extracted from Chlorella sp. NITT 05 and Picochlorum sp.
NITT 04 by DMBA is more sustainable in terms of both lipid and solvent recovery. In addition to the
tertiary amines, secondary amines such as 2-Methylaminoethanol, 2-Ethylaminoethanol,
Diisopropylamine and Diethylamine were also checked for its ability to extract lipids. But, all the
secondary amines listed above formed a monophasic system (amine and microalgal sample as a single
phase) instead of biphasic system (amine and microalgal sample as different phase) as observed in
tertiary amines, thereby leading to difficulty in separating lipids from amines and also in switching from
hydrophobic to hydrophilic and vice-versa. Hence, lipid extraction from wet microalgal suspension was
achieved through tertiary amine as switchable solvent. For switching from hydrophobic state to
hydrophilic state, all the three tertiary amines were purged with CO\(_2\). After CO\(_2\) purging, the switching of
hydrophilic tertiary amine to hydrophobic amine differed for each of the tertiary amines used. For DMBA,
hydrophilic to hydrophobic state was achieved through N\(_2\) purging. For DMCHA, it was achieved through
constant stirring and heating at 80\(^\circ\)C. For DIPEA, switching was difficult as it led to formation of salts
after exposure to CO\(_2\). During lipid extraction, amines establish interaction with the available lipid
molecules preferably after disruption of cell wall. Neutral lipids on the other hand form globules in the
cytoplasm as they are of long hydrocarbon chains and when amine enters the cell, it forms vanderwaals
interaction with neutral lipids and transfers from the cell through concentration gradient \(^{20}\). Solvents
generally work by the principle of ‘like dissolves like’. Amines with adequate ‘alkyl’ groups behave as non-
polar solvents. In switchable solvent system, amines react with CO\(_2\) to form corresponding carbamic
acids as an intermediate and further gets converted to corresponding carbamate salts. At this point of
time, amines behave as polar solvents; upon triggering by bubbling argon or nitrogen gas, it reverts to its
original form \(^{21}\). The reaction between amine and CO\(_2\) is understood by zwitterion mechanism. In this
mechanism, amines, which are basic in nature reacts with acidic CO\(_2\) gas and forms an intermediate
zwitterion consisting of pool of both the charges. In a consecutive step, the protons from the zwitter ion
intermediate transfers to a second molecule known as a base and becomes protonated, thus leaving
carbamate ions, where base can be amine, hydroxyl ions, water or alcohol. Another well-known
mechanism is intermolecular reaction mechanism which takes place in a single step. Amines, base, and
CO\(_2\) react together in a single step without any intermediate formation, resulting in carbamate ions and
protonated base \(^{22}\).

\[
\begin{align*}
NR_3 + H_2O & \xrightleftharpoons[\text{N}_2]{} [NR_3H^+][HCO_3^-] \\
\end{align*}
\]
In case of tertiary amines, bicarbonate salts which are soluble in water are formed when exposed to \( \text{CO}_2 \). The switching of tertiary amine is illustrated by the following reaction, Eq. (1). In this regard, it is noteworthy to mention that, DMBA is determined to be an efficient and competent tertiary amine for microalgal lipid extraction in terms of extraction performance, and easy switching (energy efficient processes like heating and stirring not required).

### 2.3.1. Solvent recovery and reuse

The tertiary amines DMBA, and DMCHA recovered after first extraction of lipid was subsequently used for extraction of lipids from the wet microalgae for the second cycle. The solvent recovery is presented in Fig. 4b and it is inferred that the overall solvent recovery for both DMBA and DMCHA is less than 30% and varied slightly between the microalgal species. Among the two tertiary amines, DMBA is considered as a potential solvent in terms of both lipid extraction and solvent recovery followed by DMCHA. In concern with DIPEA, it was difficult to recover the amines after first extraction and therefore, the recovered solvents of DMBA (23–28%) and DMCHA (25–30%) were used for second cycle of extraction. Though the solvent recovered was in minimal quantity, it retains its lipid extracting efficacy as the lipid content extracted from fresh and recovered solvent was similar as evident from Fig. 4a.

### 2.4. TLC for qualitative estimation

Qualitative determination of lipid types present in switchable solvent extracted total lipids was carried out. To ensure the presence of neutral lipids in DMBA, DMCHA, and DIPEA extracted total lipids, TLC was carried out. Further, only one strain i.e., *Picochlorum* sp. NITT 04 was chosen for TLC experimentation as TLC refers to be qualitative separation and therefore, lipids of all strains were subjected for GC analysis to quantify the fatty acid composition. As shown in Fig. 5, the presence of neutral lipid (*Picochlorum* sp.) in TLC is encircled in all solvent types used. Based on i) the presence of neutral lipid ii) solvent recovery and iii) lipid content and (iv) switchability phenomenon, DMBA is considered as ideal solvent for lipid extraction.

### 2.5. Fatty acid compositional analysis of microalgae

The predominant fatty acids present in *Chlorella* sp. NITT02, *Chlorella* sp. NITT05, and *Picochlorum* sp. NITT04 was presented in Table 2. Among the fatty acids, the 16 carbon long chain palmitic acid is the found to be predominant at about 24.77, 29.4, and 28.32% in *Chlorella* sp. NITT 02, *Chlorella* sp. NITT 05, and *Picochlorum* sp. NITT 04, respectively. Palmitic acid is one of the major saturated fatty acids which are commonly found in many microalgal species. The difference among the fatty acid dominance for all the three strains was clearly visualized based on color-coding in Heat map (Fig. 6). The second most predominant fatty acid present in tested strain was oleic acid (C18:1), which accounts 14.4, 17.7, and 18.5%, respectively for *Chlorella* sp. NITT 02, *Chlorella* sp. NITT 05, and *Picochlorum* sp. NITT 04 among the total fatty acid composition. In fact, many studies report the same type of fatty acids to be the major component in the total FAME content. As graphically represented in heat map, of the strains analyzed for FAME, *Chlorella* sp. NITT 02 possesses high levels of polyunsaturated fatty acids (PUFA).
such as linoleic acid, linolenic acid, and Cis-13,16- docosodienoic acid, which contributes 27% in total fatty acid composition of the strain. In contrast, *Chlorella* sp. NITT 05 accumulates saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) maximally. It is noteworthy to say that the presence of higher amount of SFAs than PUFAs makes the biodiesel less incline towards rancidification. In the case of *Picochlorum* sp. NITT 04, it is interesting to note that, monounsaturated fatty acids were present in higher concentration along with lower levels of PUFAs. These results are similar to the studies conducted by El-kassas, (2014) and Yang et al., (2015) where high amounts of MUFAs are observed than the polyunsaturated fatty acids for marine *Picochlorum* sp. The single double bond containing fatty acid types namely oleic acid and palmitoleic acid accounts at about 18.5 and 8.5%, respectively in *Picochlorum* sp. of this study and further, linolenic acid content was estimated to be less than 12%. This fatty acid content is almost similar to the fatty acid content of *Picochlorum oklahomensis* where 13.85%, 8.2%, and 13.52% of oleic acid, palmitoleic acid, and linolenic acid respectively were observed. From Table 2, it is understood that maximal concentration of SFAs and MUFAs and minimal concentration of PUFAs were observed in both *Chlorella* sp. NITT 05 and *Picochlorum* sp. NITT 04. Likewise, 32.8% of SFAs and 62.3% of total monosaturated and disaturated fatty acids were observed in *Picochlorum* sp. under ambient conditions. Fatty acid composition decides the fuel properties of biodiesel. The fuel properties to be assessed for using biodiesel as a fuel are oxidative stability, cetane number, viscosity, cold filter plugging point or low temperature property, flash point, pour point, ash content, total glycerol content, calorific value etc. Higher concentration of SFAs is beneficial for biodiesel as they determine the oxidative stability of the fuel and higher SFA content is linearly proportional to oxidatively stable biodiesel. Also, higher cetane number (CN) is obtained by the presence of high level of SFAs where CN determines the ignition potential i.e., higher CN, less time for ignition and vice versa. At the same time, presence of linolenic acid greater than 12% causes the CN to be very low, thus making the quality of biodiesel to be at low standard. The FAME profile of the tested microalgal species in this study satisfies the above-mentioned properties of biodiesel standard. On the other hand, high level of SFAs increases the viscosity as well as raise Cold filter plugging point (CFPP) thus, claiming the biodiesel to be unsuitable to operate under low temperature. Overall, there should be optimal balance of saturated and unsaturated fatty acids to qualify the properties listed for biodiesel standard. Autooxidation of the fuel relies upon the double bond present in the fatty acids. Increase in the double bond numbers (PUFA) in fatty acids makes the biodiesel susceptible to autooxidation. Compared to allylic positions, bis-allylic positions in the fatty acid chain are susceptible to autooxidation. And it is widely known that linoleic acid contains one bis-allylic position and linolenic acid contains two bis-allylic position which is why their concentration in the total FAME content is supposed to be less.
Table 2
Fatty acid methyl ester composition of *Chlorella sp. NITT02, Chlorella sp. NITT05, Picochlorum sp. NITT04*

| Fatty acids                  | Carbon number and double bond of fatty acids | Percentage of fatty acids (%) in total FAME pool |
|------------------------------|---------------------------------------------|-----------------------------------------------|
|                              |                                             | Chlorella sp. NITT02 | Chlorella sp. NITT05 | Picochlorum sp. NITT04 |
| Lauric acid                  | C12:0                                       | 5.98             | 6.470            | 3.230                 |
| Tridecanoic acid             | C13:0                                       | 3.94             | 4.030            | 3.120                 |
| Myristic acid                | C14:0                                       | Lower conc.       | Lower conc.      | 3.280                 |
| Palmitic acid                | C16:0                                       | 24.77            | 29.430           | 28.320                |
| Palmitoleic acid             | C16:1                                       | 8.87             | 10.670           | 8.550                 |
| Oleic acid                   | C18:1                                       | 14.43            | 17.710           | 18.500                |
| Linoleic acid                | C18:2                                       | 10.65            | 4.090            | 3.920                 |
| Linolenic acid               | C18:3                                       | 12.05            | 11.450           | 9.130                 |
| Cis-11,14-eicosodienoic acid | C20:2                                       | 3.28             | 4.150            | 3.570                 |
| Cis-11,14,17-eicosatrienoic acid | C20:3                                     | 3.55             | 3.180            | 4.180                 |
| Cis-13,16-docosodienoic acid | C22:2                                       | 4.34             | 3.080            | 3.440                 |
| Tricosonoic acid             | C23:0                                       | 3.76             | 3.110            | 3.030                 |
| Fatty acids detected in trace concentration | -                                          | 4.38             | 2.63             | 7.710                 |

3. Conclusion

The present study showed the potential of tertiary amine to be used as green solvent in microalgal lipid extraction amidst the conventional solvents available for lipid extraction. Among the tertiary amines used, DMBA exhibited better extraction efficiency than DMCHA. Direct use of microalgal suspension without dewatering and recycling of solvent for subsequent lipid extractions in this study portrays green energy from sustainable source. Moreover, the FAME profile of individual marine microalgal species elucidated the prospects of being a good biodiesel. Overall, this study imparts the use of tertiary amines as switchable solvent for cost and energy effective lipid extraction from microalgae.
4. Materials And Methods

4.1. Strain collection and maintenance

Marine microalgal samples collected from three different sites across the South-East coast of India were brought to the laboratory and isolated through conventional purification methods. The unialgal and axenic microalgal strains were inoculated in ASN-III medium in a thermostatically controlled environment under the specified conditions of 25ºC ± 2ºC with 1500 lux light intensity. All the experimental cultures were maintained at artificial illumination of 16 h light: 8 h dark photoperiod.

4.2. Growth assessment

To harvest excessive amount of biomass from microalgae, growth profile of the strains needs to be known. Optical density was taken as growth metrics in this study. Biomass density of cultures was determined from 0th day to day 27 spectrophotometrically based on the absorbance (Optical density) at 750 nm as there is no chlorophyll interference at this wavelength.

4.3. Switchable solvents for lipid extraction

Switchable solvents used for lipid extraction are tertiary amines, which includes DMCHA (Dimethycyclohexylamine), DMBA (Dimethylbenzylamine) and DIPEA (Diisopropylethylamine). The methodology of tertiary amine as switchable solvent for wet lipid extraction from marine microalgae is illustrated in Fig. 2. In brief, known volume of microalgal suspension taken directly from the culture flask was used for lipid extraction without drying, which was ultrasonicated for rupturing the cells. To this, 1:1 (v/v) of tertiary amines (DMCHA, DMBA and DIPEA) was added which formed two immiscible layers. It was then subjected to constant stirring for 1 hour at 1000 rpm to allow the tertiary amine to extract lipids from ruptured microalgal cells. After this, CO₂ was purged to enable switchability and lipid in top layer was collected and the hydrophilic amines were switched to its native (hydrophobic) state by purging N₂. Eventually, the recovered SPS was used for another cycle of lipid extraction. The lipids extracted were dried and expressed in %.

4.3.1. Qualitative analysis of lipids extracted by tertiary amines

The total lipid extracted by each of the tertiary amines (DMCHA), DMBA and DIPEA) was qualitatively analysed for different classes of lipids by Thin Layer Chromatography (TLC). Silica gel coated plate was used as a stationary phase and the solvent mixture in the ratio of 70:30:1 (Hexane: Diethylether: Acetic acid) was used as mobile phase. The solute and TAG standard (Triolein) were loaded and run in parallel. The presence of the solute spot was compared with the standard lipid and the lipid classes were separated and visualized by Iodine vapour and Charring method. For visualization through charring method, TLC plates were sprayed with 10% CuSO₄ in 8% orthophosphoric acid solution and kept for charring at 180ºC for few minutes.
4.4. FAME production from Switchable solvent extracted lipids

Conversion of lipid to FAME was carried out based on our previous work\textsuperscript{34}. In brief, homogeneous acid catalysis using methanolic sulphuric acid (3.5\%) was used for fatty acid methylation; 65 °C and 2.5 h was set as reaction temperature and reaction time, respectively. At the end of the reaction, the crude or unpurified FAME contents were allowed for the separation of ester and glycerol and the upper FAME layer was collected and purified with water wash. The purified FAME was dissolved in hexane for its profile analysis.

4.4.1. Gas chromatographic conditions for FAME analysis

FAME composition of switchable solvent extracted lipids were analysed on gas chromatography (Perkin Elmer Clarus 500) coupled to SP 2560 capillary column (100 m length) and detector-FID. Nitrogen as carrier gas with flow rate – 1 mL min\(^{-1}\) was used. The oven temperature-140 °C – 240 °C, injection port and detector temperature- 260 °C and 45 min total run time are the operating conditions of the GC. The peak area of the fatty acids was compared with the peak area of Supelco 37 component FAME mix standard to ascertain and quantify each fatty acid analyte.

4.5. Statistical analysis

For heat map plotting, “R” language has been used through R studio package. All the experiments were carried out in triplicates to obtain reproducible data with accuracy. Positive and negative deviation of the triplicate experiments was recorded.

Declarations

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Authors Contribution statement

Susaimanickam Anto is the main author who carried out all the experiments and data analysis, M. Premalatha helped in interpretation of data and editing, Thangavel Mathimani directed the investigation, design of work and wrote the original manuscript. All authors have unanimously approved the manuscript.

Competing Interests
The authors declare no competing interests.

References

1. Mathimani, T. & Mallick, N. A comprehensive review on harvesting of microalgae for biodiesel - Key challenges and future directions. *Renew. Sustain. Energy Rev.* **91**, (2018).

2. Bai, X. *et al.* Proteomic analyses bring new insights into the effect of a dark stress on lipid biosynthesis in Phaeodactylum tricornutum. *Sci. Rep.* **6**, 1–10 (2016).

3. Mathimani, T., Kumar, T. S., Chandrasekar, M., Uma, L. & Prabaharan, D. Assessment of fuel properties, engine performance and emission characteristics of outdoor grown marine Chlorella vulgaris BDUG 91771 biodiesel. *Renew. Energy* **105**, 637–646 (2017).

4. Lau, P.-C., Kwong, T.-L. & Yung, K.-F. Effective heterogeneous transition metal glycerolates catalysts for one-step biodiesel production from low grade non-refined Jatropha oil and crude aqueous bioethanol. *Sci. Rep.* **6**, 23822 (2016).

5. Liang, M.-H., Qv, X.-Y., Jin, H.-H. & Jiang, J.-G. Characterization and expression of AMP-forming Acetyl-CoA Synthetase from Dunaliella tertiolecta and its response to nitrogen starvation stress. *Sci. Rep.* **6**, 23445 (2016).

6. Al Ahmad, M., Al-Zuhair, S., Taher, H. & Hilal-Alnaqbi, A. RF Microalgal lipid content characterization. *Sci. Rep.* **4**, 5108 (2014).

7. Slocombe, S. P. *et al.* Unlocking nature’s treasure-chest: screening for oleaginous algae. *Sci. Rep.* **5**, 9844 (2015).

8. Mathimani, T., Uma, L. & Prabaharan, D. Optimization of direct solvent lipid extraction kinetics on marine trebouxiophycean alga by central composite design – Bioenergy perspective. *Energy Convers. Manag.* **142**, 334–346 (2017).

9. Du, Y., Schuur, B., Kersten, S. R. A. & Brilman, D. W. F. Opportunities for switchable solvents for lipid extraction from wet algal biomass: An energy evaluation. *ALGAL* **11**, 271–283 (2015).

10. Samor, C., Galletti, P., Pasteris, A. & Tagliavini, E. Synthesis of new polyethoxylated tertiary amines and their use as Switchable Hydrophilicity. *RSC Adv.* **4**, 5999–6008 (2014).

11. Zhu, Y. & Turgut, N. Growth and Biomass Characteristics of Picochlorum oklahomensis and Nannochloropsis oculata. *J Am Oil Chem Soc* **90**, 841–849 (2013).

12. Chioccioli, M., Hankamer, B. & Ross, I. L. Flow cytometry pulse width data enables rapid and sensitive estimation of biomass dry weight in the microalgae Chlamydomonas reinhardtii and Chlorella vulgaris. *PLoS One* **9**, 1–12 (2014).

13. Nawkarkar, P., Singh, A. K., Abdin, M. Z. & Kumar, S. Life cycle assessment of Chlorella species producing biodiesel and remediating wastewater. *J. Biosci.* **44**, 1–15 (2019).

14. Rajapitamahuni, S., Bachani, P., Sardar, R. K. & Mishra, S. Co-cultivation of siderophore-producing bacteria Idiomarina loihiensis RS14 with Chlorella variabilis ATCC 12198, evaluation of micro-algal growth, lipid, and protein content under iron starvation. *J. Appl. Phycol.* **31**, 29–39 (2019).
15. El-Sheekh, M., Abu-Faddan, M., Abo-Shady, A., Nassar, M. Z. A. & Labib, W. Molecular identification, biomass, and biochemical composition of the marine chlorophyte Chlorella sp. MF1 isolated from Suez Bay. *J. Genet. Eng. Biotechnol.* **18**, (2020).

16. Thangavel, K. *et al.* Growth and metabolic characteristics of oleaginous microalgal isolates from Nilgiri biosphere Reserve of India. *BMC Microbiol.* **18**, 1–17 (2018).

17. Boyd, A. R. *et al.* Bioresource Technology Switchable hydrophilicity solvents for lipid extraction from microalgae for biofuel production. *Bioresour. Technol.* **118**, 628–632 (2012).

18. Samorí, C. *et al.* Effective lipid extraction from algae cultures using switchable solvents. *Green Chem.* **15**, 353–356 (2013).

19. Phan, L. *et al.* Switchable-Polarity Solvents Prepared with a Single Liquid Component. *J. Org. Chem* **73**, 127–132 (2008).

20. Huang, W. & Kim, J. Simultaneous cell disruption and lipid extraction in a microagal biomass using a nonpolar tertiary amine. *Bioresour. Technol.* **232**, 142–145 (2017).

21. Plaumann, H. Switchable Polarity Solvents: Are They Green? Abstract: *Phys. Sci. Rev.* 27–30 (2017). doi:10.1515/psr-2016-0073

22. Yuksel orhan, O., Ozturk, M. C., Seker, A. & Alper, E. Kinetics and performance studies of a switchable solvent TMG. *Turkish J. Chem.* **39**, 13–24 (2015).

23. Chen, M. *et al.* Subcritical co-solvents extraction of lipid from wet microalgae pastes of Nannochloropsis sp. *Eur.J.Lipid Sci.Technol* **114**, 205–212 (2012).

24. Battah, M., Abomohra, A. E. & Esmael, A. Optimization of Growth and Lipid Production of the Chlorophyte Microalg Chlorella vulgaris as a Feedstock for Biodiesel Production Optimization of Growth and Lipid Production of the Chlorophyte Microalga. *World Appl. Sci. J.* **28**, 1536–1543 (2013).

25. Yao, L., Gerde, J. A., Lee, S. & Wang, T. Microalgae Lipid Characterization. *J. Agric. Food Chem.* **63**, 1773–1787 (2015).

26. El-kassas, H. Y. Growth and fatty acid profile of the marine microalga Picochlorum Sp. grown under nutrient stress conditions. *Egypt. J. Aquat. Res.* **39**, 233–239 (2014).

27. Yang, F. *et al.* Extracting Lipids from Several Species of Wet Microalgae Using. *Energy & Fuels* **29**, 2380–2386 (2015).

28. Moser, B. R. Influence of Blending Canola, Palm, Soybean, and Sunflower Oil Methyl Esters on Fuel Properties of Biodiesel Influence of Blending Canola, Palm, Soybean, and Sunflower Oil Methyl Esters on Fuel Properties of Biodiesel †. *Energy & Fuels* **22**, 4301–4306 (2008).

29. Deshmukh, S., Bala, K. & Kumar, R. Selection of microalgae species based on their lipid content, fatty acid profile and apparent fuel properties for biodiesel production. *Environ. Sci. Pollut. Res.* (2019).

30. Nascimento, I. A. *et al.* Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. *Bioenergy Res.* **6**, 1–13 (2013).
31. Ambat, I., Bec, S., Peltomaa, E., Srivastava, V. & Ojala, A. A synergic approach for nutrient recovery and biodiesel production by the cultivation of microalga species in the fertilizer plant wastewater. Sci. Rep. 9, 1–9 (2019).

32. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. J. Gen. Microbiol. 111, 1–61 (1979).

33. Katsuki, Y., Yamaguchi, Y. & Tani, M. Overexpression of PDR16 confers resistance to complex sphingolipid biosynthesis inhibitor aureobasidin A in yeast Saccharomyces cerevisiae. FEMS Microbiol. Lett. 365, 1–10 (2018).

34. Mathimani, Uma, L. & Prabaharan, D. Homogeneous acid catalysed transesterification of marine microalga Chlorella sp. BDUG 91771 lipid - An efficient biodiesel yield and its characterization. Renew. Energy 81, 523–533 (2015).