First Report on Tertiary Amine as a CO_2 Switchable Solvent for Hypersaline Trebouxio phycean Microalgae Towards Greener and Competent Lipid Extraction

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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 sector³. In this scenario, biodiesel is clearly emerging as an alternative supplement, which can be readily, introduced into the existing transportation infrastructure without engine modification⁴. Though, many feedstocks are being used for biodiesel, microalgae hold great promise as a hopeful production platform for biodiesel⁵ 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 technique⁸. 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, CO₂ switchable solvent systems for lipid extraction would indeed be a great choice. CO₂ 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^{9,10}. 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.

Table 1
Marine microalgal isolates and their geographical sites in South East Coast, India

Isolate No.	Strain name	Habitat	Collection site	GPS
Isolate – 2	<i>Chlorella</i> sp. NITT 02	Saltpan	Kanyakumari, Tamil Nadu	8° 6'38.91"N 77°29'13.43"E
Isolate – 4	<i>Picochlorum</i> NITT 04	Stagnant seawater	Kanyakumari Tamil Nadu	8° 7'28.10"N 77°29'16.89"E
Isolate – 5	<i>Chlorella</i> sp. NITT 05	Stagnant seawater	Muttukaddu, Chennai	12° 48' 45. 76" N 80° 14' 37. 67" E

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 time¹¹. 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 OD₇₅₀ 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^{13–16}. 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 an 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 *Picochlorum* 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 yield¹⁷. Another study by Samorì 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. NITT05 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 CO₂. After CO₂ 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 N₂ purging. For DMCHA, it was achieved through constant stirring and heating at 80°C. For DIPEA, switching was difficult as it led to formation of salts after exposure to CO₂. 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 CO₂ 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 CO₂ is understood by zwitterion mechanism. In this mechanism, amines, which are basic in nature reacts with acidic CO₂ 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 CO₂ react together in a single step without any intermediate formation, resulting in carbamate ions and protonated base ²².

(1)

In case of tertiary amines, bicarbonate salts which are soluble in water are formed when exposed to CO₂. The switching of tertiary amine is illustrated by the following reaction ,Eq. (1) ⁹. 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 ^{15,23–25}. 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 PUFA. 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 ¹¹. 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 ²⁷. 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 ²⁸. 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 ²⁹. 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 ³⁰. 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 autoxidation. 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 ³¹.

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- eicosotrienoic 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 medium³² in a thermostatically controlled environment under the specified conditions of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ 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, CO_2 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 N_2 . 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% CuSO_4 in 8% orthophosphoric acid solution and kept for charring at 180°C for few minutes³³.

4.4. FAME production from Switchable solvent extracted lipids

Conversion of lipid to FAME was carried out based on our previous work³⁴. 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.

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Figures

Figure 1

Asynchronous growth profile of tested microalgal strains over cultivation days (0-27 days). Optical density at 750 nm as growth metrics for (a) *Chlorella* sp. NITT02 (b) *Chlorella* sp. NITT05 (c) *Picochlorum* sp. NITT04. Initial cell density of all the cultures was fixed at ~0.6 OD.

Figure 2

Tertiary amine as switchable solvent for wet lipid extraction from marine microalgae

Biphasic system before CO₂ purging

**Monophasic system of amine and media
after CO₂ purging**

Figure 3

Switchability nature of tertiary amine during lipid extraction

a **b** **Figure 4**

(a) Different tertiary amines on the lipid content of microalgae. The amount of lipid extracted by fresh tertiary amine was indicated as “fresh” and the amount of lipid extracted by the solvent after first use was indicated as “recovered”, (b) Percentage of DMBA and DMCHA solvents recovery after their first used in lipid extraction

Figure 5

TLC of total lipids extracted from *Picochlorum* sp. by tertiary amines (Charring method)

Figure 6

Heat map of the fatty acid composition of the tested strains