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Lipid Extraction by Ozonolysis from Scenedesmus Sp. Followed by Purification Using Column Chromatography

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Abstract

Microalgae are gaining attention as a promising source of various bioproducts, ranging from biofuels to essential food components, due to their potential in multi-product biorefineries. They offer several advantages over traditional food crops, such as higher biomass productivity, reduced land requirements, and the ability to grow on non-arable land. The extraction of their lipid content can be achieved through more eco-friendly options, such as ozonolysis and methanol. These lipids can then be utilized for biodiesel production through esterification or for the consumption of essential fatty acids. This study focused on extracting lipids from Scenedesmus sp. biomass using methanol with the assistance of ozonolysis, followed by purification with adsorbents in a chromatographic column. Results show that product formation in the ozonation extraction process, microalgae lipid extraction, and yields (Ozonation for 20 minutes at 20°C, 40 minutes at 40°C, and 60 minutes at 60°C). Among the ozonation of the mixture for 20 minutes + 20°C produced several compounds (2-pentadecanone, 6, 10, 14-trimethyl; n-hexadecanoic acid (also known as palmitic acid); phytol and octadecanoic acid. Ozonation for 40 minutes +40°C, and 60 minutes +60°C clearly produced 3 main compounds (2 pentadecanone, 6, 10, 14-trimethyl; palmitic acid; stearic acid,).

1. INTRODUCTION

Microalgae exhibit several advantages compared to terrestrial plants, as they do not compete with conventional food production and are not lignocellulosic biomass. Their biomass consists mainly of three primary metabolites: proteins, carbohydrates, and lipids, which can be used to derive many bio products; however, the most studied application is biofuel production from the lipid fraction. Microalgae, which are important feedstocks for third- and fourth-generation biofuels, can grow in seawater, wastewater, food waste, and even saline–alkaline soil, and can obtain higher biomass than land plants using limited nutrients (Maeda et al., 2017, Lynch et al., 2015, Wang et al., 2021, Tuz et al., 2018, Chong et al., 2021). Microalgae exhibit a fast growth rate, higher photosynthetic efficiency, and better land utilization rates, which can yield a range of biofuel products (Maliha and Abu-Hijleh, 2022; Kovacevic and Wesseler, 2010). In the past two decades, scientists have made significant strides in researching microalgae biodiesel. Several species, including Chlorella vulgaris, Nannochloropsisoceanica, Dunaliellsalina, Botryococcus, Desmodesmus, Neochloris, Scenedesmus and Tetraselmis, have been identified as suitable for biodiesel production.

Hence, biofuel production is receiving global attention and holds promising prospects for development and future demand. Besides,TheBP Statistical Review of World Energy 2024 report highlights record-high global energy consumption of 620 Exajoules (up 2%), and record-high fossil fuel consumption and energy-related CO2 emissions, despite a substantial surge in renewable electricity generation. Key findings include a 13% increase in renewable electricity (excluding hydro), record oil consumption of over 100 million barrels per day, and significant coal consumption growth in India. Simultaneously, there is a continuous emergence of new national-level policies encouraging the gradual substitution of fossil fuels with biofuels (Aziz et al., 2013, Soccolet al., 2005). As one of the developing countries, India faces the problem of fossil fuels shortage and carbon emission. In recent years, India is also striving to find a sustainable development path of energy, and the biofuel industry is considered to be an important means to ensure energy security. However, as their large-scale production is still not competitive for this biofuel, an increasing number of researchers have focused on improving biomass productivity and lowering costs and energy demand in all stages.Based on the findings, the lipid extraction by ozonation can possibly bypass the needs of energy-intensive pre-treatment methods such as microwave, bead mills, osmotic pressure, autoclaving, electroporation, and ultrasonication

which previously have been reported to improve the efficiency of the solvent-extraction process (Garoma and Janda, 2016). Moreover, ozonation extraction process might be the solution to the solvent diffusion limitation and lipids polarity problems faced due to the high water content in solvent extraction process (Fajardo et al., 2007).

2. MATERIALS AND METHODS

2.1 isolation of Microalgae

In this study, a comprehensive collection of 116 samples from various terrestrial and aquatic habitats was collected in the Ariyalur District, with geographical coordinates of Latitude 11.1503 and Longitude 79.0685. The terrestrial samples, each weighing approximately 100 grams, were collected in polybags and mixed thoroughly, while aquatic samples of around 100 mL were gathered in blue-capped containers. All samples were stored at a controlled temperature of 4°C in a refrigerator for preservation.

To isolate various microalgal species, Sterilized Bold Basal medium (BBM) was employed. The process began with enrichment culture methods aimed at obtaining unialgal colonies of microalgae. Culturing was performed in a controlled environment characterized by a light intensity of 3.5 klux, following a 16:8 light-dark cycle, at a temperature of 28±2°C, and with the pH of the medium adjusted to 8.0 to optimize growth conditions. The BBM was autoclaved and dispensed into 250 ml conical flasks, with 1 ml of collected water samples introduced into each. These flasks were then incubated for a period of 10-15 days. After this incubation, significant green growth of algal cells was observed, which were collected using Pasteur pipettes to isolate the unialgal cells.

The isolated unialgal cells were subsequently re-cultured in another 100 ml conical flask, again containing sterilized BBM, and incubated for an additional 10-15 days. For the maintenance of the cultures, a solid agar-based medium was prepared by dissolving 15-18 g of purified agar in 1 liter of the medium, followed by autoclaving. Plates containing this medium were also incubated for 10-15 days, allowing the isolated colonies to grow. The microalgal strains isolated from the selected habitats were cultivated continuously in BBM under similar controlled conditions. To ensure culture purity, cultures were routinely streaked on plates with suitable solid media and were examined through microscopic observation at regular intervals. Discrete colonies were then inoculated in fresh media and utilized for further study during their exponential growth phase.

2.2 Screening

Among 116 microalgal isolates, twenty species were identified, encompassing Aphanothece sp., Phormidium sp., Aulosira sp., Gloecapsa sp., Lyngbya sp., Chlorella sp., Chroococcus sp., Nostoc sp., Nostoccalcasie sp., Oscillatoria sp., Microcoleus sp., Botryococcus sp., Chlorococcum sp., Chlamydomonas sp., and Scenedesmus sp. These species are classified into two algal taxa: Chlorophyta (green algae) and Cyanobacteria (blue-green algae). Cyanobacteria included Aphanothece sp., Phormidium sp., Aulosira sp., Gloecapsa sp., Lyngbya sp., Botryococcus sp., Chroococcus sp., Nostoc sp., Nostoccalcasie sp., Oscillatoria sp., and Microcoleus sp. Meanwhile, Chlorophyta consisted of Chlorella sp., Scenedesmus sp., Chlamydomonas sp., and Chlorococcum sp. To assess algal biomass production and lipid accumulation strategies, the study began by isolating a potent microalgal strain suitable for biofuel. After purifying and cultivating the twenty identified strains under axenic conditions in BG-11 medium, the first experiment measured the growth rates of all strains, revealing irregular but continuous growth patterns. A follow-up experiment evaluated lipid content under controlled growth conditions. Promising strains were selected based on growth rates and lipid accumulation, with Scenedesmus sp. being further cultivated under varying conditions to explore its lipid accumulation strategy. This detailed assessment lays the groundwork for potential biofuel applications from microalgal sources.

2.3 Microalgal biomass

Microalgal biomass was produced by cultivating the ScendesmusSp in liquid BG-11 medium under standardized cultural and environmental conditions. A 1 ml aliquot of homogenized algal suspension was inoculated into each flask on a laminar flow bench, properly labeled, and incubated for durations of 15 days, 22 days, and 29 days. All isolates were cultivated in triplicate to analyze biomass and lipid content, and the mean of the three replicates was calculated to obtain average estimates of biomass weight (g/l) and lipid percentage. The data were subjected to statistical analysis to perform ANOVA and estimate the standard error

of the mean. The harvested biomass was dried in an oven at 60°C for 48 hours, was subsequently utilized for lipid quantification.

2.4 Lipid quantification

The examined algal species was conducted using a modified version of the gravimetric technique established by Bligh and Dyer, as detailed by Storms et al. (2014). Approximately 40 mg of dry algal biomass was weighed and transferred into a mortar that had been prewashed with hexane. The biomass was ground for 5 minutes using a pestle. Hexane was then added to the mortar, and the resulting slurry was homogenized. The mixture was centrifuged (Remi RM 03) for 20 minutes at 10,000 rpm. The supernatant was pipetted into a pre-weighed metal weighing dish and placed in a fume hood to allow for the evaporation of hexane. The mass of the extracted oil was determined gravimetrically after the hexane had completely evaporated. The mass of the extracted oil was then expressed as a percentage of the dry cell weight (dcw) of the algal biomass.

2.5 Ozonolysis

In the present study possible disruption mechanism in Scendesmussp cells were carried out. The cell membranes of Scendesmus cells are first attacked by ozone and unsaturated lipids and protein present in the membrane serve to be the prime target. A reaction called oxidative burst occurred when the ozone molecule contacted with the cell membrane which creates a tiny hole resulting the cell to lose it shape. The presence of methanol will further disrupt and extract the internal lipids.

Lipids were extracted using methanol with dry biomass and organic solvent at the mass: volume ratio of 1:10. Residual biomass was filtered and solvent containing extracted lipids was evaporated in a rotary evaporator (IKA RV 10). The extraction yield was calculated considering the mass of lipids in the dry biomass. The development of the lipid extraction process by direct ozonolysis in ozonation – extraction bioreactor. The harvested algal slurries were mixed with methanol during the extraction process in different ozonationtimesi.e 20 Mintues +20˚C, 40 Mintues +40˚C and 60 Mintues +60˚C temperature were tested.

2.6 Lipid purification

After extraction using methanol and the solvent evaporation, a purification step was conducted using adsorbent material activated carbon packed in a chromatographic column. Extracts were purified using a typical chromatographic column packed with silica gel and an adsorbent as stationary phase, and chloroform as mobile phase. Firstly, the column was packed with the stopcock closed and chloroform was carefully added to uniformly wet all adsorbent materials. After opening the stopcock, the extracted lipid (around 1-2 g) was dissolved in a few ml of chloroform and added through the top of the column. Chloroform was added drop wise until elution was complete. The end of purification was established when an evident green colour reached the bottom of the chromatographic column, which indicates the elution of pigments the same column after elution with chloroform until pigments reached the bottom part. The reported purification yield in this step is the recovered lipids from the column.

2.7 Lipid extracts characterization

Lipid extracts were evaluated qualitatively by the colour of the eluted solvent and lipids mixture, and quantitatively by GC-MS acylglycerols quantification. Based on ASTM D6584-17 and EN 14105, the crude lipids extracted from dry biomass were analysed to quantify FFA, MAG, DAG, and TAG.The purified lipids after purification were also analysed. This quantification method was employed using a GC FID (Agilent 7890A) and two internal standards (tricaprin and 1,2,4-butanetriol). AOCS Official Method Ca 12 55 (AOCS 2009) was performed to determine phosphorus or the equivalent phosphatide content on microalgae lipids. This method applies originally to crude, degummed, and refined vegetable oils, by a shaking the sample in the presence of ZnO, followed by the spectrophotometric measurement of P as a blue phosphomolybdic acid complex. A calibration curve was obtained for potassium dihydrogen phosphate.

3. RESULTS AND DISCUSSION

The present results show that product formation in the ozonation extraction process, microalgae lipid extraction, and yields (Ozonation for 20 minutes at 20˚C, 40 minutes at 40˚C, and 60 minutes at 60˚C). The color of approximately the same mass of lipids dissolved in chloroform after the step of ozonation, assisted by methanol extraction and purification using activated charcoal. The samples were analysed by GC-MS and the main compounds detected were compared with NIST database. Among the ozonation of the mixture for 20 minutes + 20˚C produced several compounds (2-pentadecanone, 6, 10, 14-trimethyl; n-

hexadecanoic acid (also known as palmitic acid); phytol and octadecanoic acid. Ozonation for 40 minutes +40°C, and 60 minutes +60°C clearly produced 3 main compounds (2 pentadecanone, 6, 10, 14-trimethyl; palmitic acid; stearic acid, Figure-1). The main compounds detected and the mechanisms involved are discussed.

Santillan-Jimenez et al. (2016) also achieved efficient removal of chlorophyll from Scenedesmusacutus crude lipids using activated carbon packed in a chromatographic column. The obtained fatty acids include C16:0 and C18:1, which are in agreement with the classification of D. salina and have been reported by many researchers (Assunção et al., 2012 and Giordano et al., 2014). Due to the sensitivity of polyunsaturated fatty acid to oxidation (Lamers et al., 2012), The accumulation of saturated fatty acid (hexadecanoic acid, octadecanoic acid) along with the time of ozonation. Lin and Hong (2013), reported that ozonation of Chlorococcumaquaticum with methanol in a sand filtration reactor generated several products in the form of long-chain largely saturated hydrocarbons with 16 to 20 carbons. They also suggested that with ozonation, the composition of biodiesel can be controlled and would be beneficial for utilization in cold regions (unsaturated hydrocarbons) or more oxidation-resistant (saturated hydrocarbons).In this part, transesterification was not performed to study the possibility of direct transesterification by ozonation. It has been reported by Lin and Hong (2013), due to elevated boiling and melting points, saturated compounds elute slowly in the GC column relative to their unsaturated compounds, resulting in their absence under conditions used for unsaturated hydrocarbons. With the setting used GC-MS method fatty acids were detected.

Efficient lipid extractions could also attenuate these challenges, such as the application of green and clean methods using supercritical CO2 (scCO2), which maintains lipid quality (Marino et al. 2021), or nonpolar solvents. This work provides new information on lipid extraction and purification by comparing different conditions and quantifying acylglycerol groups. Few studies have performed micro algal oil purification using chromatographic columns packed with adsorbent materials. Richmond and Hu (2013) used a column with silicon dioxide, silica gel, and anhydrous sodium sulfate to elute neutral lipids (hydrocarbons, pigments, sterols, triglycerides, waxes, etc.) with chloroform, followed by fatty acids and polar lipids (glycolipids, phospholipids) with methanol.

Similar results observed the accumulation of saturated fatty acid (hexadecanoic acid, octadecanoic acid) along with the time of ozonation. Lin and Hong (2013), reported that, transesterification was not performed to study the possibility of direct transesterification by ozonation. saturated compounds elute slowly in the GC column relative to their unsaturated compounds, resulting in their absence under conditions used for unsaturated hydrocarbons.

The present study produced low concentrations of hexadecanoic acid and phytol which are due to minimal breakage of the cells during separation process (solvent and centrifugation). Phytol is an acrylic diterpene alcohol which originates from chlorophyll 132 metabolism and is used in industries as a fragrance agent (flowery odor) (Yamamoto et al., 2014). The increase of 2-pentadecanone, 6, 10, 14-trimethyl, which has been previously reported in Scenedesmus and Chlorella vulgaris cells extracted by steam distillation (Rzama et al., 1995), could be due to the degradation of higher hydrocarbon compounds.

Similar efficient lipid extractions could also attenuate these challenges, such as the application of green and clean methods using supercritical CO2 (scCO2), which is maintain lipid quality (Marino et al. 2021: Ritabrata et al. 2024), or nonpolar solvents. This work provides new information on lipid extraction and purification by comparing different conditions and quantifying acylglycerol groups. Few studies have performed microalgal oil purification using chromatographic columns packed with adsorbent materials. Richmond and Hu (2013) used a column with silicon dioxide, silica gel, and anhydrous sodium sulfate to elute neutral lipids (hydrocarbons, pigments, sterols, triglycerides, waxes, etc.) with chloroform, followed by fatty acids and polar lipids (glycolipids, phospholipids) with methanol. Santillan Jimenez et al. (2016) used columns packed with silica gel and activated carbon to elute neutral lipids with diethyl ether. Finally, In contrast, Lorenzen et al. (2017) also used a column packed with bentonite but with n-hexane. Based on these studies and a vast literature on lipid extraction.

Similar reports the pressure-assisted ozonation (PAO) methods have been reported to disrupt 80.3% of Chlorella cells and to produce 24% (w/w) of lipid yield (Huang et al., 2016), other routes such as ultrasonication and supercritical CO2 also require large amounts of energy: about 110% and 66% respectively

of the total biodiesel production energy (Brentner et al. 2011). This study showed an efficient lipid purification method that can be applied to microalgae crude oil, obtaining a clean product and potentially avoiding catalyst poisoning during the oil conversion into more valuable products. However, greener solvent alternatives should be explored to avoid the use of toxic solvents. Nonetheless, there are still very limited research on this topic, which should be further investigated as microalgae are promising biomass.

4. CONCLUSION

This study focuses on optimizing total lipid extraction from the microalga Scenedesmus sp. through the process of ozonation. The proposed method is noted for its practicality and cost-effectiveness, making it suitable for application in research laboratories, as it requires a low-cost and low-power ozonation unit. While the study advocates for a univariate approach compared to existing literature, the highlighted multivariate optimization is particularly beneficial as it significantly reduces the number of experiments needed. This reduction leads to decreased waste of time, energy, and reagents. Furthermore, despite the fatty acid profile being rich in unsaturated compounds, the study asserts that biodiesel production is feasible, corroborating findings from previous research.

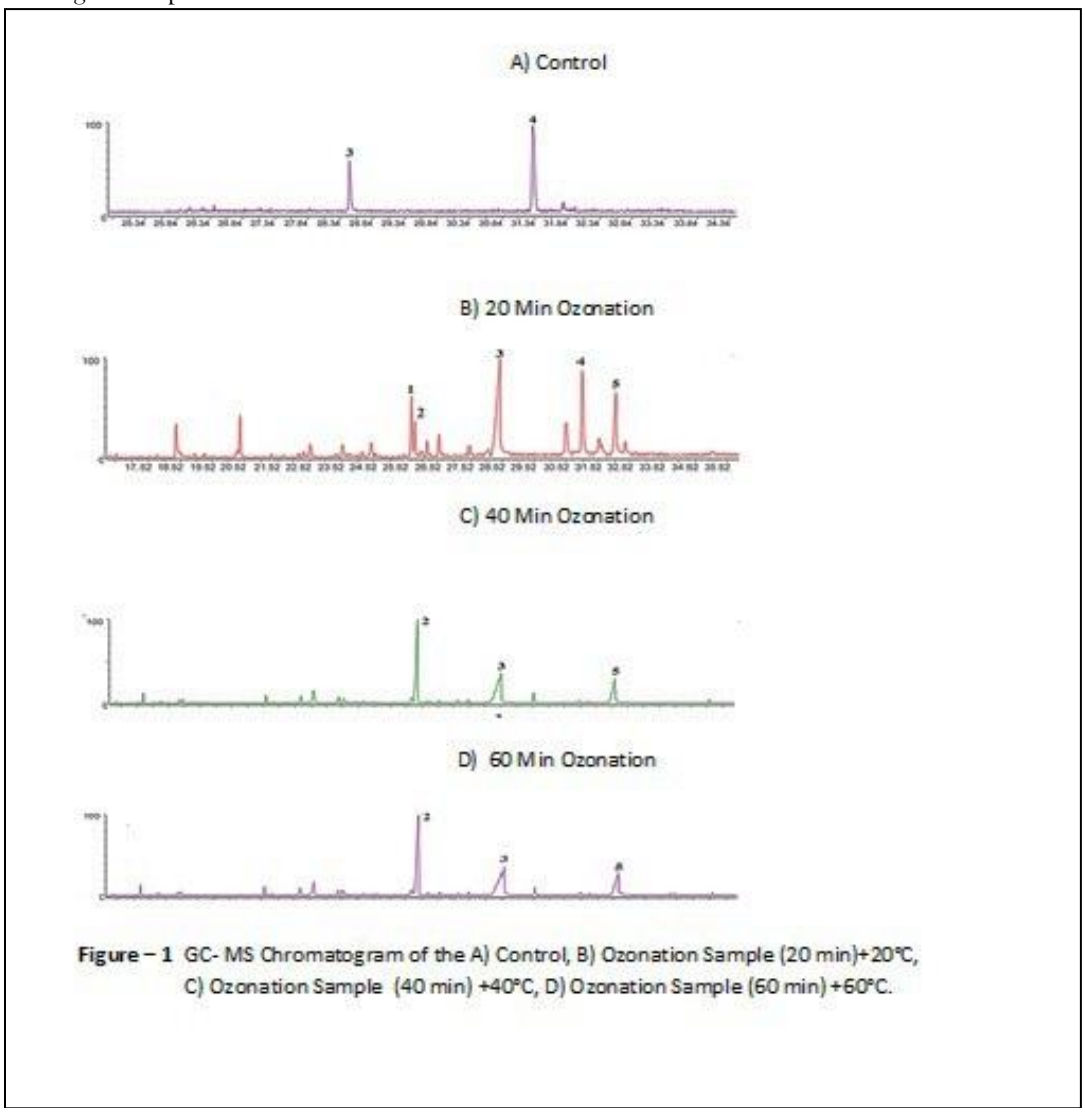

Figure – 1 GC- MS Chromatogram of the A) Control, B) Ozonation Sample (20 min)+20°C, C) Ozonation Sample (40 min) +40°C, D) Ozonation Sample (60 min) +60°C.

5. **REFERENCE:**

1. Assunção, P., Jaén-Molina, R., Caujapé-Castells, J., de la Jara, A., Carmona, L., Freijanes, K. and Mendoza, H. (2012) 'Molecular taxonomy of Dunaliella (Chlorophyceae), with a special focus on D. salina: ITS2 sequences revisited with an extensive geographical sampling', Aquatic Biosystems, 8(1), p. 2.

2. Aziz E, Anna S, Pascal L. Biofuels and the sustainability challenge: a global assessment of sustainability issues, trends and policies for biofuels and related feedstocks. Rome, Italy: Food And Agriculture Organization of The United Nations. 2013.
3. Bischoff, H.W. & Bold, H.C. (1963). Phycological studies IV. Some soil algae from Enchanted Rock and related algal species. University of Texas Publication 6318: [1]-95, 145 f.
4. Bold, H.C. (1949). The morphology of Chlamydomonaschlamydogama, sp. nov.. Bulletin of the Torrey Botanical Club 72(2): 101-108.
5. BP Statistical Review of World Energy. 2024.
https://www.energyinst.org/__data/assets/pdf_file/0006/1542714/684_EI_Stat_Review_V16_DIGITAL.pdf .June 20, 2024.
6. Brentner, L. B., M. J. Eckelman and J. B. Zimmerman. 2011. Combinatorial Life Cycle Assessment to Inform Process Design of Industrial Production of Algal Biodiesel. Environ. Sci. Technol. 45(16): 7060-7067.
7. Chong JWR, Yew GY, Khoo KS, Ho S-H, Show PL. Recent advances on food waste pretreatment technology via microalgae for source of polyhydroxyalkanoates. J Environ Manage. 2021;293: 112782.
8. Fajardo, A. R., Cerdán, L. E., Medina, A. R., Fernández, F. G. A., Moreno, P. a. G. and Grima, E. M. (2007) 'Lipid extraction from the microalga Phaeodactylumtricornutum', European Journal of Lipid Science and Techno ogy, 109(2), pp.120–126.
9. Garoma T, Janda D. Investigation of the effects of microalgal cell concentration and electroporation, microwave and ultrasonication on lipid extraction efficiency. Renew Energ. 2016;86:117–123. doi: 10.1016/j.renene.2015.08.009.
10. Giordano, M., Bargnesi, F., Mariani, P. and Cryptogamie, S. (2014) 'Dunaliellasalina (Chlorophyceae) Affects the Quality of NaCl Crystals', Cryptogamie, Algologie, 35(3), pp. 285–302.
11. Fajardo, A. R., Cerdán, L. E., Medina, A. R., Fernández, F. G. A., Moreno, P. a. G. and Grima, E. M. (2007) 'Lipid extraction from the microalga Phaeodactylumtricornutum', European Journal of Lipid Science and Techno ogy, 109(2), pp.120–126.
12. Kovacevic V, Wesseler J. Cost-effectiveness analysis of algae energy production in the EU. Energy Policy. 2010;38(10):5749–57.
13. Lamers PP, Janssen M, De Vos RCH, Bino RJ, Wijffels RH. Carotenoid and fatty acid metabolism in nitrogen-starved Dunaliellasalina, a unicellular green microalga. J Biotechnol. 2012;162(1):21–7.
14. Lin, C. C. and Hong, P. K. A. (2013) 'A new processing scheme from algae suspension to collected lipid using sand filtration and ozonation', Algal Research, 2(4), pp.378–384.
15. Lorenzen, J., Igl, N., Tippelt, M., Stege, A., Qoura, F., Sohling, U., Brück, T. 2017. 'Extraction of Microalgae Derived Lipids with Supercritical Carbon Dioxide in an Industrial Relevant Pilot Plant'. Bioprocess and Biosystems Engineering 40 (6): 911–18.
16. Lynch F, Santana-Sanchez A, Jamsa M, Sivonen K, Aro E-M, Allahverdiyeva Y. Screening native isolates of cyanobacteria and a green alga for integrated wastewater treatment, biomass accumulation and neutral lipid production. Algal Res. 2015;11:411–20.
Maeda Y, Yoshino T, Matsunaga T, Matsumoto M, Tanaka T. Marine microalgae for production of biofuels and chemicals. CurrOpinBiotechnol. 2017;50:111–20.
Maliha A, Abu-Hijleh B. A review on the current status and postpandemic prospects of third-generation biofuels. Energy Syst. 2022;14:1–32.
17. Marino, T., Leone, G. P., Casella, P., Iovine, A., Musmarra, D., Zoani, C., Balducchi, R., Molino, A.. 2021. 'Green Extraction of Microalgae Components for Incorporation in Food and Feed Supplements'. Chemical Engineering Transactions 87 (July): 457–62.
18. Plohn M, Spain O, Sirin S, Silva M, Escudero-Onate C, Ferrando-Climent L, Allahverdiyeva Y, Funk C. Wastewater treatment by microalgae. Physiol Plant. 2021;173:568–78.
19. Richmond, A., Qiang, H., eds. 2013. Handbook of Microalgal Culture: Applied Phycology and Biotechnology. Second edition. Chichester, West Sussex, UK: John Wiley & Sons, Ltd.
20. RitabrataKoley, BelaraniMahato,KuntalHazra, AfsonaKhatun, RajshekharGhorai, AnupamAdhikari, BiplabDebnath and Shaileyee Das.Microalgae Chlorella Vulgaris isolation and GCMS Analysis of PhytocomponentMethanolicExtracts Journal of Propulsion Technology Vol. 45:4369 - 4375 (2024)
21. Rzama A., Benharref A., Arreguy B., Dufourc E. J. (1995). Volatile compounds of green microalgae grown on reused waste water. Phytochem 38, 1375–1379. 10.1016/0031-
22. Santillan-Jimenez, E., Pace, R., Marques, S., Morgan, T., McKelphin, C., Mobley, J., Crocker, M. 2016. 'Extraction, Characterization, Purification and Catalytic Upgrading of Algae Lipids to Fuel-like Hydrocarbons'. Fuel 180 (September): 668–78.
23. Soccol CR, Vandenberghe LPS, Costa B, Woiciechowski AL, Carvalho JCD, Medeiros ABP, Francisco AM, Bonomi LJ. Brazilian biofuel program an overview. J SciIndust Res. 2005;64(11):897–904.
24. Storms, Z. J., Cameron, E., de la HozSiegler, H., McCaffrey, W. C. A Simple and Rapid Protocol for Measuring Neutral Lipids in Algal Cells Using Fluorescence. J. Vis. Exp. (87), e51441, doi:10.3791/51441 (2014).
25. Tu Z, Liu L, Ln W, Xie Z, Luo J. Potential of using sodium bicarbonate as external carbon source to cultivate microalga in non-sterile condition.Bioresour Technol. 2018;266:109–15.
26. Wang M, Liu H, Qiao K, Ye X, Takano T, Liu S, Bu Y. Exogenous NaHCO3 enhances growth and lipid accumulation of the highly NaHCO3-tolerant Nannochlorissp. JB17. J ApplPhycol. 2021;33:241–53.
27. Yamamoto, M., Baldermann, S., Yoshikawa, K., Fujita, A., Mase, N. and Watanabe, N. (2014) 'Determination of volatile compounds in four commercial samples of Japanese green algae using solid phase microextraction gas chromatography mass spectrometry', The Scientific World Journal, Article ID 289780, 8 pages