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# Extraction and GCMS Characterization of Chlorella vulgaris Microalgal Oil Using n-Hexane Assisted Dissolution Method

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**Abstract:** Microalgae-based biodiesel has emerged as a promising third-generation biofuel feedstock, yet efficient downstream lipid recovery remains a critical bottleneck. This study investigated the effectiveness of n-hexane-assisted dissolution in improving the recovery and homogeneity of microalgal oil extracted from *Chlorella vulgaris* biomass, and characterized the fatty acid methyl ester (FAME) composition of the recovered oil using Gas Chromatography-Mass Spectrometry (GC-MS). Two dissolution methods were evaluated: direct dissolution (Variation 1) and n-hexane-assisted dissolution (Variation 2), each at volumetric ratios of 1:4 and 1:2 (v/v). GC-MS analysis of the base microalgal oil identified nine FAME compounds with carbon chain lengths ranging from C15 to C21, with methyl nonadecanoate ($C_{19}H_{38}O_2$) as the dominant constituent at 50.63% of the total chromatographic area and a total methyl ester content of 93.23%. In Variation 1, total methyl ester content reached 67.41% (Sample A) and 80.70% (Sample B), with sediment formation observed in both samples. In contrast, Variation 2 achieved markedly higher total methyl ester contents of 98.40% (Sample 1) and 98.25% (Sample 2), with no sediment detected, indicating superior lipid dispersion and product homogeneity. The predominance of C17–C19 fatty acid methyl esters across all samples confirms the suitability of *Chlorella vulgaris* oil as a renewable biofuel feedstock. These results demonstrate that n-hexane-assisted dissolution is an effective strategy for enhancing microalgal oil recovery and highlight the critical role of solvent selection in downstream lipid processing for microalgae-based biofuel production.

**Keywords:** *Chlorella vulgaris*, GC-MS, lipid extraction, n-hexane dissolution, fatty acid methyl ester

## INTRODUCTION

The world's growing population, coupled with accelerating industrial activities, has driven an unprecedented surge in global energy consumption. To date, fossil fuels remain the dominant source meeting this demand, contributing substantially to greenhouse gas emissions

and intensifying the adverse effects of climate change. In response, the pursuit of sustainable and environmentally friendly renewable energy alternatives has emerged as a critical agenda on the global stage (Tripathi et al., 2025). Beyond reducing carbon emissions, renewable energy technologies offer the added benefit of strengthening energy security by reducing reliance on depleting fossil fuel reserves (Henni et al., 2026).

Among the renewable energy options under investigation, biofuels stand out as a promising candidate for substituting petroleum-based fuels. Nevertheless, the conventional route of biodiesel production heavily depends on edible vegetable oils including palm, soybean, and sunflower oil, raising concerns over food-fuel competition and the large-scale land use required for their cultivation (Shaima et al., 2022). Such drawbacks have motivated the scientific community to seek more sustainable and non-competing feedstock alternatives for biofuel production (Kumar, 2026).

Microalgae have attracted considerable attention as one of the most prospective third-generation biofuel feedstocks, owing to their fast growth rate, high photosynthetic efficiency, and inherent capacity to assimilate carbon dioxide as a carbon source. Moreover, microalgae can be cultivated on marginal, non-arable land without competing with food crop production. Relative to terrestrial oilseed crops, microalgae demonstrate markedly superior lipid productivity per unit cultivation area, positioning them as highly attractive candidates for renewable fuel development (Thanigaivel et al., 2022).

Of the numerous microalgal species investigated, *Chlorella vulgaris* has been extensively studied for its broad adaptability to varying environmental conditions, rapid biomass accumulation, and considerable lipid content. Under optimized growth conditions, the lipid content of *Chlorella vulgaris* may reach as high as 58% of dry biomass weight, underscoring its substantial potential as a feedstock for microalgal oil and biodiesel production (Johnson et al., 2025; Tok et al., 2023). Despite this potential, the economic viability of microalgal biofuel production remains heavily contingent on the efficiency of downstream processing steps, particularly lipid extraction and recovery (Gupta et al., 2021; Sreenikethanam et al., 2023).

Lipid extraction is widely regarded as one of the most pivotal and cost-intensive stages in microalgal biorefinery systems. The robust cell wall architecture of *Chlorella vulgaris* impedes solvent penetration and restricts the release of intracellular lipids, thereby reducing overall extraction efficiency. Consequently, the design of effective extraction strategies is indispensable for maximizing lipid recovery and enhancing the commercial viability of microalgal biofuel production (Kanaga et al., 2022; Rajpoot et al., 2025).

Among the solvents commonly employed for lipid extraction, n-hexane is particularly favored owing to its high selectivity toward nonpolar lipids, low boiling point, and relatively straightforward solvent recovery. Prior investigations have established that solvent polarity is a key determinant of lipid extraction efficiency and fatty acid recovery from microalgal biomass (Ferdous et al., 2023; Zarrinmehr et al., 2022). Nevertheless, the effectiveness of n-hexane-assisted dissolution as a means of enhancing microalgal oil recovery specifically from *Chlorella vulgaris* biomass has yet to be thoroughly elucidated.

In light of these considerations, the present study aims to investigate the effectiveness of n-hexane-assisted dissolution in improving microalgal oil recovery from *Chlorella vulgaris* biomass. In addition, the extracted oil was further characterized by Gas Chromatography-Mass Spectrometry (GC-MS) analysis to determine its fatty acid methyl ester (FAME) composition and assess its potential as a renewable biofuel feedstock

## METHOD

### Materials

The primary material used in this study was microalgal oil derived from Chlorella vulgaris biomass obtained from laboratory cultivation. The recovered oil was used as the

feedstock for dissolution experiments and subsequent chemical characterization. Analytical-grade n-hexane was employed as the dissolution solvent due to its strong affinity toward nonpolar lipid compounds and its widespread application in microalgal lipid recovery processes (Davani et al., 2022; Parimalachelvam et al., 2023). Distilled water was used during sample preparation and cleaning procedures.

**Preparation of Microalgal Oil**

The microalgal oil utilized in this study was obtained from Chlorella vulgaris biomass harvested after cultivation. Before analysis, the recovered oil was concentrated using a water bath operated at temperatures below 70°C to minimize thermal degradation of fatty acid compounds and preserve lipid quality during sample preparation (Kumar, 2026; Lima-Pereira et al., 2025). The concentrated oil was then stored in sealed containers at room temperature until further use.

**Dissolution Methods**

The effectiveness of oil dissolution was evaluated using two different approaches. The experimental design was intended to compare direct dissolution and n-hexane-assisted dissolution in terms of product homogeneity and fatty acid methyl ester recovery. Solvent-assisted dissolution has been reported as an effective approach for enhancing lipid transfer and improving the recovery of nonpolar lipid fractions from microalgal biomass (Oliva et al., 2024; Ramesh & Thiyagarajan, 2023).

**Variation 1: Direct Dissolution Method**

In the direct dissolution method, microalgal oil was directly mixed with the receiving medium at volumetric ratios of 1:4 and 1:2 (v/v). The mixtures were continuously stirred until a visually uniform dispersion was obtained. The resulting samples were designated as Sample A (1:4) and Sample B (1:2). The presence of sediment formation was recorded as an indicator of incomplete dissolution

**Variation 2: n-Hexane-Assisted Dissolution Method**

In the n-hexane-assisted dissolution method, microalgal oil was first dissolved in n-hexane to improve lipid transfer and dispersion. The mixture was stirred thoroughly until a homogeneous solution was achieved. Following dissolution, n-hexane was removed using a water bath operated at temperatures below 70°C. The resulting samples were prepared at volumetric ratios of 1:4 and 1:2 and subsequently analyzed. Visual observations were conducted to evaluate the occurrence of sediment formation and phase separation.

**Homogeneity Evaluation**

The dissolution performance of each method was assessed through visual examination of sediment formation and phase stability. Samples exhibiting no visible sediment were considered to possess superior homogeneity and improved lipid dispersion characteristics. The observations were recorded and compared between dissolution methods.

**GC-MS Analysis**

The chemical composition of the recovered microalgal oil was determined using Gas Chromatography-Mass Spectrometry (GC-MS), a widely used analytical technique for identifying and quantifying fatty acid methyl esters in microalgal biofuel studies (Kiani et al., 2022). Compound identification was carried out by comparing the obtained mass spectra with the National Institute of Standards and Technology (NIST) spectral database following established analytical procedures (Wallace & Moorthy, 2023; Zapata-Boada et al., 2022). The relative abundance of each compound was calculated based on chromatographic peak area

percentages. Particular attention was given to the identification of fatty acid methyl esters (FAMEs) due to their importance in evaluating the biofuel potential of microalgal oil.

## RESULTS AND DISCUSSION

### Fatty Acid Composition of Chlorella vulgaris Microalgal Oil

The chemical composition of Chlorella vulgaris microalgal oil was characterized using Gas Chromatography-Mass Spectrometry (GC-MS) to identify and quantify the fatty acid methyl ester (FAME) components present in the recovered oil. GC-MS is widely recognized as a reliable analytical technique for separating and identifying complex organic compounds in microalgal biofuel feedstocks with high accuracy and sensitivity (Kaeoboon et al., 2025; Pantami et al., 2020). The GC-MS chromatogram of the recovered microalgal oil is presented in Figure 1.

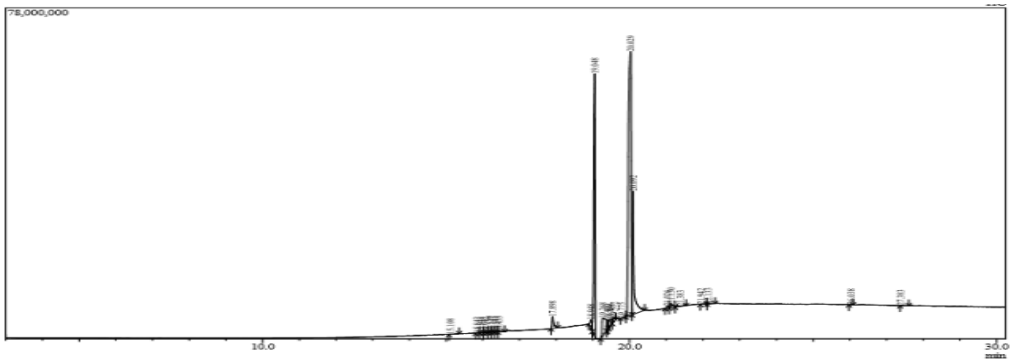

**Figure 1.** GC-MS chromatogram of recovered Chlorella vulgaris microalgal oil

The chromatogram revealed the presence of nine methyl ester compounds with carbon chain lengths ranging from C15 to C21, as summarized in Table 1.

**Table 1.** Composition of Biodiesel Methyl Esters (GC-MS)

| No. | Molecular Formula | Function Group | % Area |
|---|---|---|---|
| 1 | $C_{15}H_{30}O_2$ | Ester | 1,32 |
| 2 | $C_{17}H_{32}O_2$ | Ester | 24,44 |
| 3 | $C_{17}H_{34}O_2$ | Ester | 1,16 |
| 4 | $C_{18}H_{34}O_2$ | Ester | 0,52 |
| 5 | $C_{18}H_{36}O_2$ | Ester | 1,45 |
| 6 | $C_{19}H_{36}O_2$ | Ester | 12,62 |
| 7 | $C_{19}H_{38}O_2$ | Ester | 50,63 |
| 8 | $C_{21}H_{40}O_2$ | Ester | 0,79 |
| 9 | $C_{21}H_{42}O_2$ | Ester | 0,30 |
| **Total Methyl Ester** | | | **93,23** |

The chromatogram revealed the presence of several methyl ester compounds with carbon chain lengths ranging from C15 to C21. The predominance of medium and long chain fatty acid methyl esters indicates that the recovered oil possesses characteristics suitable for renewable biofuel applications. Similar carbon chain distributions have been reported in microalgal oils intended for biodiesel production, where fatty acids in the C15-C21 range contribute significantly to fuel quality, ignition performance, and oxidative stability (Kim et al., 2025; Singh et al., 2024).

Among the identified compounds, methyl nonadecanoate ($C_{19}H_{38}O_2$) was found to be the dominant constituent, accounting for 50.63% of the total chromatographic area. The dominance of long-chain fatty acid methyl esters suggests a substantial accumulation of neutral lipids

within the recovered oil. Long-chain methyl esters are known to enhance cetane number and improve combustion characteristics, making them desirable components for biofuel production (Verma et al., 2024; Zhu et al., 2022).

The total methyl ester content of the recovered oil reached 93.23%, indicating that the extracted microalgal oil consisted predominantly of lipid-derived compounds. This result confirms the potential of Chlorella vulgaris as a promising feedstock for renewable energy applications and supports previous findings regarding its ability to accumulate significant quantities of biodiesel-relevant fatty acids under suitable cultivation conditions (Zapata-Boada et al., 2022; Zhu et al., 2022).

**Effect of Direct Dissolution Method on Microalgal Oil Recovery (Variation 1)**

The effectiveness of the direct dissolution method was evaluated using two volumetric ratios, namely 1:4 (Sample A) and 1:2 (Sample B). The GC-MS chromatograms obtained from both samples are presented in Figures 2 and 3, respectively, while the identified compounds are summarized in Table 2.

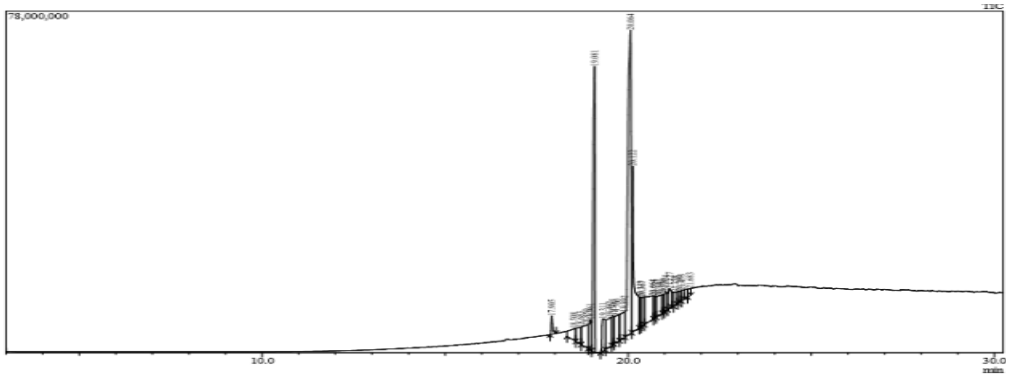

**Figure 2.** GC-MS chromatogram of Chlorella vulgaris microalgal oil obtained from the direct dissolution method (Sample A, 1:4 v/v ratio)

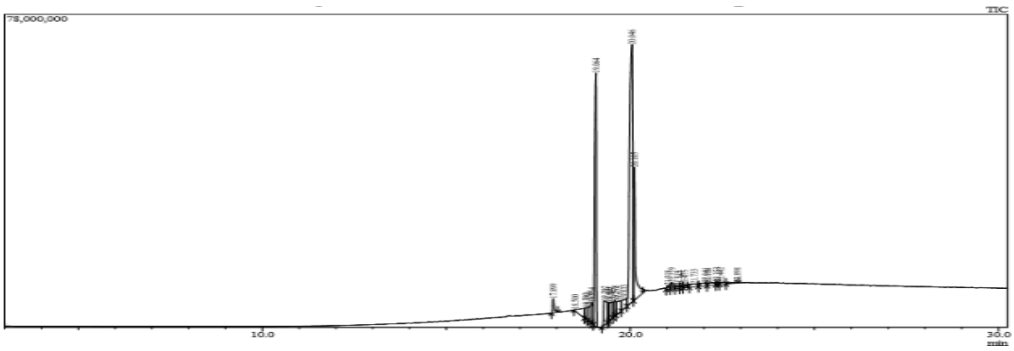

**Figure 3.** GC-MS chromatogram of Chlorella vulgaris microalgal oil obtained from the direct dissolution method (Sample B, 1:2 v/v ratio)

**Table 2.** Composition of Methyl Ester Fatty Acids in Microalgae-Biodiesel Solution Variation 1 (% Area)

| Sample | C15:0 | C16:0 | C17:0 | C17:1 | C18:0 | C18:1 | C19:0 | C19:1 | C21:0 | C21:1 |
|---|---|---|---|---|---|---|---|---|---|---|
| **A (1:4)** | 0,78 | 1,99 | 14,3 | 2,45 | 1,68 | 4,52 | 10 | 28,6 | 1,6 | 1,49 |
| **B (1:2)** | 1,2 | 2,1 | 20,5 | 2,47 | 1,75 | 2,86 | 10 | 38,5 | 0,85 | 0,47 |

Sample A exhibited a total methyl ester content of 67.41%, whereas Sample B showed a higher value of 80.70%. The increase observed in Sample B suggests that a higher proportion of microalgal oil in the dissolution system facilitated greater retention of lipid-derived compounds within the recovered phase. However, visual observations revealed the presence of sediment in both samples, indicating incomplete dissolution and limited dispersion of certain lipid fractions.

The occurrence of sediment formation suggests that direct dissolution alone was insufficient to completely transfer and distribute the lipid components throughout the system. This phenomenon may be attributed to the limited interaction between nonpolar lipid molecules and the surrounding medium, resulting in phase instability and reduced recovery efficiency. Similar observations have been reported in studies investigating microalgal lipid recovery, where inadequate solvent interaction often leads to incomplete dissolution and lower extraction performance (Yasin et al., 2023; Zapata-Boada et al., 2022).

Although the direct dissolution method was capable of recovering a substantial fraction of fatty acid methyl esters, the relatively lower methyl ester content and the presence of sediment indicate that additional solvent assistance may be required to improve lipid transfer and product homogeneity.

**Effect of n-Hexane-Assisted Dissolution on Microalgal Oil Recovery (Variation 2)**

To improve lipid dispersion and recovery, n-hexane-assisted dissolution was applied in Variation 2. The resulting chromatograms for Samples 1 and 2 are shown in Figures 4 and 5, while the corresponding compound compositions are summarized in Table 3

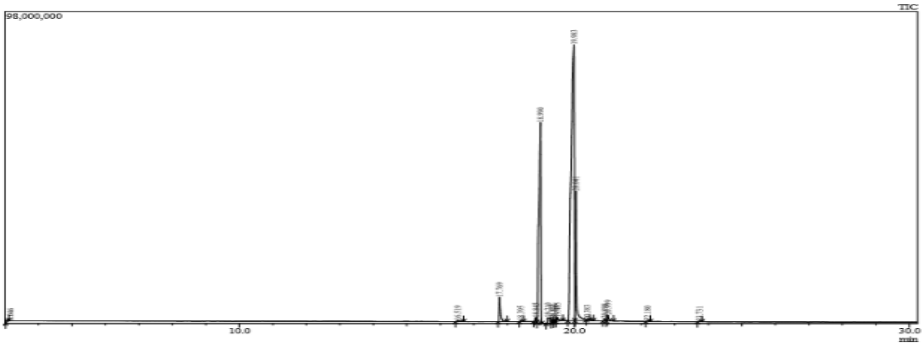

**Figure 4.** GC-MS chromatogram of Chlorella vulgaris microalgal oil obtained from the n-hexane-assisted dissolution method (Sample 1, 1:4 v/v ratio)

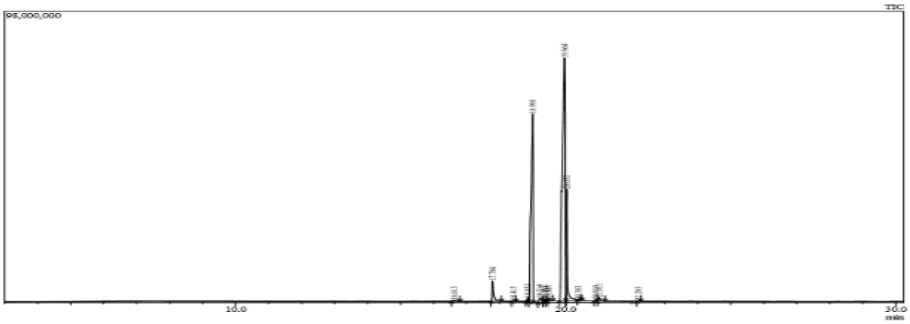

**Figure 5.** GC-MS chromatogram of Chlorella vulgaris microalgal oil obtained from the n-hexane-assisted dissolution method (Sample 2, 1:2 v/v ratio)

**Table 3.** Composition of Methyl Ester Fatty Acids of Microalgae-Biodiesel Solution Variation 2 (% Area)

| Sampel | C15:0 | C17:0 | C18:0 | C18:1 | C19:0 | C19:1 | C23:0 |
|---|---|---|---|---|---|---|---|
| **1 (1:4)** | 2,43 | 31,04 | 0,62 | 0,25 | 9,12 | 54,94 | 0,07 |
| **2 (1:2)** | 2,15 | 26,53 | 0,92 | 0,52 | 9,51 | 58,54 | 0,08 |

The total methyl ester content obtained using the n-hexane-assisted dissolution method reached 98.40% for Sample 1 (1:4 ratio) and 98.25% for Sample 2 (1:2 ratio). These values were substantially higher than those observed in the direct dissolution method, indicating that n-hexane significantly enhanced the recovery and distribution of lipid-derived compounds within the system.

Unlike Variation 1, no sediment formation was observed in either sample treated with n-hexane. The absence of sediment indicates improved homogeneity and more complete dispersion of lipid fractions throughout the dissolution medium. This observation suggests that n-hexane effectively facilitated the transfer of intracellular neutral lipids and fatty acid methyl esters into the liquid phase.

The superior performance of n-hexane can be attributed to its nonpolar characteristics, which provide strong interactions with triglycerides, neutral lipids, and long-chain fatty acids commonly present in *Chlorella vulgaris* oil. Previous studies have demonstrated that nonpolar solvents such as n-hexane exhibit high affinity toward hydrophobic lipid compounds, thereby improving extraction efficiency and increasing lipid recovery yields (Devi et al., 2024; Kiani et al., 2022).

The consistently high methyl ester content obtained in both samples demonstrates that n-hexane-assisted dissolution is an effective strategy for enhancing microalgal oil recovery and improving the quality of the resulting lipid product.

**Comparison of Dissolution Methods and the Role of n-Hexane**

A comparison of the two dissolution methods is presented in Table 4. The direct dissolution method produced total methyl ester contents ranging from 67.41% to 80.70%, whereas the n-hexane-assisted dissolution method achieved values between 98.25% and 98.40%.

**Table 4.** Comparison of Total Methyl Ester of Both Solubility Variations

| Variation | Sample | Ratio (v/v) | Total ME (%) | Describtion |
|---|---|---|---|---|
| **Variation 1** | A | 01:04 | 67,41 | Precipitate observed |
| **Variation 1** | B | 01:02 | 80,70 | Precipitate observed |
| **Variation 2** | 1 | 01:04 | 98,40 | Homogeneous, no precipitate |
| **Variation 2** | 2 | 01:02 | 98,25 | Homogeneous, no precipitate |

The substantial improvement observed in Variation 2 highlights the important role of n-hexane in facilitating lipid transfer and improving dissolution efficiency. As a nonpolar solvent, n-hexane possesses a strong affinity for neutral lipids and fatty acid methyl esters, enabling more effective solubilization and distribution of these compounds within the oil phase. Consequently, the recovery of methyl ester compounds increased significantly compared with the direct dissolution approach.

Furthermore, the elimination of sediment formation in all n-hexane-treated samples indicates enhanced phase stability and improved product homogeneity. In contrast, the direct dissolution method exhibited visible sediment formation, suggesting incomplete transfer of

lipid fractions and reduced system stability. These findings are consistent with previous reports demonstrating that solvent polarity is one of the most influential factors affecting lipid recovery efficiency from microalgal biomass (Arabian, 2022).

The predominance of C17-C19 fatty acid methyl esters across all samples further confirms the suitability of *Chlorella vulgaris* as a renewable biofuel feedstock. Fatty acids within this carbon-chain range are associated with favorable fuel properties, including higher cetane numbers, improved combustion efficiency, and enhanced oxidative stability (Verma et al., 2024). Therefore, the application of n-hexane-assisted dissolution not only improves lipid recovery but also supports the production of high-quality microalgal oil for sustainable biofuel applications.

## CONCLUSION

This study demonstrated that n-hexane-assisted dissolution is an effective approach for improving the recovery and homogeneity of Chlorella vulgaris microalgal oil. The enhanced dissolution performance facilitated a more efficient transfer of lipid derived compounds into the oil phase, resulting in a higher recovery of fatty acid methyl esters compared with the direct dissolution method. GC-MS characterization confirmed that the recovered oil was dominated by medium- and long-chain fatty acid methyl esters, indicating its suitability as a renewable biofuel feedstock. These findings highlight the importance of solvent-assisted dissolution in downstream microalgal oil processing and contribute to the development of more efficient lipid recovery strategies for microalgae-based biofuel production. The study also provides scientific insight into the role of solvent selection in improving the quality and recovery of microalgal oil, thereby supporting the advancement of sustainable bioenergy technologies.

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