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New Type of Extraction Solvent for Algal Oils: Fatty Acid Methyl Esters

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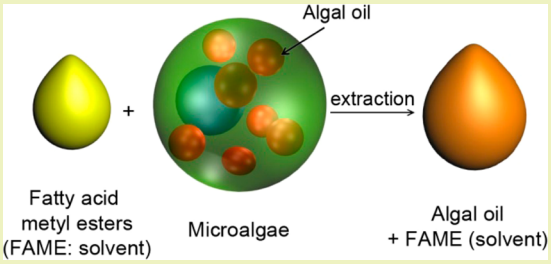
Supporting Information

ABSTRACT: We propose a new process of biodiesel production that utilizes biodiesel as an extractant for lipid extraction from fresh microalgae. Biodiesel-based extractant showed high lipid extraction efficiency from dried *Chlorella*. The efficiency obtained from the biodiesel-based extractant was comparable to the chloroform/methanol method, while the required amount of solvents was smaller than the chloroform/methanol method. Furthermore, fatty acid methyl esters displayed successful penetration through the cell membrane of wet algae in an aqueous environment, and this result suggests that the biodiesel-based extractant has potential for wet extraction.



KEYWORDS: *Microalgae, Lipid extraction, Fatty acid methyl ester, Algal oil*

INTRODUCTION

The fatty acid methyl esters (FAMEs) of bio-oil and biodiesel have been widely studied for sustainable and renewable energy in concern of diminishing fossil fuel supplies and environmental issues. Among various biomasses, microalgae have attracted considerable interest because of their hoped for rapid growth, high oil content, superior productivity per cultured area, carbon sequestration, biodegradability, and nontoxicity of algae biodiesel.[1−4] In general, algae biodiesel is produced by cultivation, harvest, oil extraction, oil refining, and trans-esterification, and each step should be organically connective within the entire process.[4,5]

Various extraction methods, such as press/expeller, solvent extraction, and supercritical fluid extraction, have been widely studied for the laboratory-scale extraction of algal oil.[5] However, the simple press/expeller technique results in inefficient extraction.[5] Furthermore, the supercritical fluid method is hampered by an expensive initial installation and utilization cost, although this technique ensures high extraction efficiency.[1,6] Meanwhile, nonpolar organic solvents, such as hexane, chloroform, and dichloroethane, have successfully extracted algal oils with or without polar solvents, such as methanol or isopropanol,[1,2] and the disruption of the strong cell walls of microalgae by pretreatment with sonication or bead beating has shown to improve the extraction efficiency.[7] However, the solvent extraction method is not recommended because it requires enormous energy consumption for oil separation and solvent recovery, although the energy might be supplied from biomass residues after lipid extraction through anaerobic digestion and power generation.[8] Therefore, development of a new extraction process that is highly efficient, cost effective, eco-friendly, and has high triglyceride selectivity is a worthwhile subject to investigate.

Herein, we propose a new process for the algal oil extraction and biodiesel production cycle using produced biodiesel as an extrantant (Figure 1 and Figure 1S, Supporting Information).

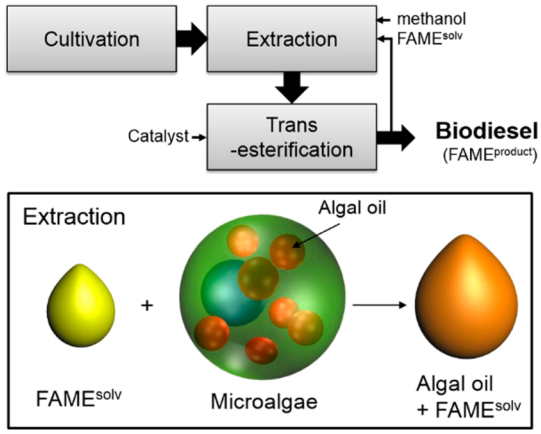


Figure 1. Simplified process drawing of biodiesel production that utilizes biodiesel-based extractant.

In this entire process, biodiesel can be recycled to produce additional algal oil from fresh feedstock. This process does not require any external solvents except for methanol, which will be utilized from the extraction through the transesterication. Furthermore, a solvent recovery process, which is the most energy intensive step in the current solvent extraction methods, is not required.[9] Therefore, the process can have competitive-

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ness in the biodiesel production in terms of economic and environmental aspects if the method can ensure high extraction efficiency. To prove whether the FAME-based solvent ($FAME^{solv}$) can be applied as an extractant, the extraction efficiency of algal oil from dried microalgae and cell membrane penetration of $FAME^{solv}$ in aqueous media were investigated using commercialized rapeseed biodiesel ($FAME^{solv}$), which has similar lipid compositions to *Chlorella* (Supporting Information 5).

EXPERIMENTAL SECTION

Extraction of Algal Oil Using $FAME^{solv}$/Methanol Extractant. Dried algae (1 or 5 g) were suspended in the $FAME^{solv}$/methanol-based extractant. The volume ratio of $FAME^{solv}$ to methanol was adjusted to 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100. Equivalent amounts of $FAME^{solv}$ (3.2 mL) containing heptanoic acid (1 vol %) was used. The suspension was stirred for 3, 5, or 12 h at 50 °C for the extraction. The mixture was filtered, and the filter cake was sequentially washed with equivalent amounts of extractant and methanol by vacuum filtration. Finally, the algal oil was washed with deionized water, and methanol was eliminated using a rotary evaporator.

Extraction of Algal Oil Using Chloroform/Methanol Extractant. The total lipids were extracted by modifying Folch's method.[10] Dried *Chlorella* or *Nannochloropsis* (10 mg) was dissolved in 2 mL of chloroform/methanol (2:1, v/v) and stirred for 12 h. The extracted oil was separated and washed with deionized water by centrifugation. Finally, *Chlorella* oil was obtained by evaporating the solvents using a rotary evaporator. It was found that 91% of oil was recovered from dried *Chlorella* (oil content = 38.5 wt %).

Transesterification of Algal Oils and GC-MS Analysis. Methanol (1 mL) was added to 2 mg of extracted oils with sulfuric acid (0.3 mL) and was stirred for 10 min. The transesterification reaction proceeded for 20 min at 100 °C under vigorous stirring. After cooling to room temperature, the organic phase was washed three times with deionized water by centrifuge-assisted separation. Fatty acid methyl esters were collected by evaporation of the solvents. The diluted FAME was analyzed by gas chromatography–mass spectrometry (QP-2010 plus, Shimadzu).

Relative extraction efficiency (%) of the $FAME^{solv}$/methanol extraction solvent against the chloroform/methanol method was calculated using eq 1 with normalized peak areas (A) based on the standard peak area of heptanoic acid.#tab;

$$\text{Relative extraction efficiency (REE) (\%)} = \frac{A_{(\text{algal oil} + FAME^{solv})} - A_{FAME^{solv}}}{A_{\text{algal oil(extracted by chloroform/methanol)}}} \times 100 \quad (1)$$

The overall extraction efficiency (%) was calculated based the percentage of peak area for each component obtained from GC-MS analysis of the chloroform/methanol extract and REE (%) for each component calculated from eq 1.

RESULT AND DISCUSSION

Miscibility of Biodiesel with Algal Oil. Author: One of the important requirements for extraction solvents is high miscibility with oils in microalgae, while the solvent is immiscible with raffinate for efficient extraction and separation.[3] $FAME^{solv}$ from bio-oil has a long alkyl chain structure that is similar to triglycerides and free fatty acids (FFAs) in microalgae. Thus, $FAME^{solv}$ is expected to have similar solubility parameters with triglycerides and FFAs. Solubility parameters for FAMEs, triglycerides, and FFA structures of hexadecanoic acid (C16:0) were calculated using the Hoftyzer–Van Krevelea method, and the calculated values were found to be 16.78, 16.05, and 17.56 $MPa^{1/2}$, respectively.[11] The three structures had similar solubility parameters and were in line with a previous report that the solubility parameters of bio-oils and biodiesel can be varied approximately 14–18 $MPa^{1/2}$.[12] It is expected that $FAME^{solv}$ is miscible with algal oils because compounds having similar solubility parameters tend to be miscible.[3,6]

Because pure $FAME^{solv}$ is viscous and is to be directly applied to the algal oil extraction process, methanol was selected as a cosolvent because of its high miscibility with $FAME^{solv}$. Furthermore, methanol can be utilized in the transesterification without being separated from the extracted lipids. As shown in Figure 2S of the Supporting Information, the viscosity of $FAME^{solv}$ exponentially decreased from ~6 to ~1 mPa s with increasing methanol content. Furthermore, methanol can dissociate lipid–protein complexes in algae cells, which improves the extraction efficiency.[13,14]

However, the addition of methanol to $FAME^{solv}$ can alter the miscibility of the solvent with oil because of the polar property of methanol, and it can result in a reduction in the extraction efficiency. Thus, the miscibility of the ternary system of $FAME^{solv}$/methanol/triglycerides or FFA was explored in various ranges of mixing ratios. The FFA of C18:1 formed a homogeneous liquid with $FAME^{solv}$/methanol in all mixing ratio ranges. The triglyceride of C18:1 (glyceryl trioleate) showed relatively high miscibility with $FAME^{solv}$/methanol, and a homogeneously miscible or phase-separated region was observed depending on the composition (Figure 2). The

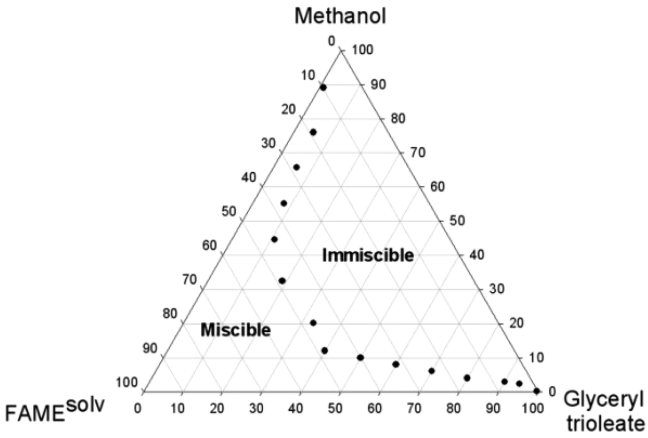

Figure 2. Phase diagram of $FAME^{solv}$/methanol/triglycerides (C18:1) ternary system. Dots denote maximum miscible points.

miscibility analysis proved the mixture of $FAME^{solv}$ and methanol at a ratio of 80:20 can dissolve the triglyceride and FFA forms of C18:1 over the entire range of compositions. Consequently, the efficiency of $FAME^{solv}$/methanol (80:20, v/v) in extracting algal oil from two species of dried microalgae was tested.

Chloroform/Methanol Solvent Extraction Method. Before testing extraction using $FAME^{solv}$, the lipid composition of dried *Chlorella* and *Nannochloropsis* were observed using the chloroform/methanol (2:1, v/v) extraction method. Produced FAMEs ($FAME^{product}$) from the transesterification reaction of the extracted lipids with methanol under sulfuric acid catalyst was analyzed by GC-MS, and the main components of *Chlorella* oil were found to be C18:1 (29%), C18:2 (28%), C16:0 (22%), C18:0 (6.5%), and C16:0 (3%) with nontargeted lipids (11.5%). However, the lipids of dried *Nannochloropsis* were mainly composed of C18:1 (32%), C16:0 (21%), C18:2 (17%),

Table 1. Summarized Relative Extraction Efficiency (%) Based on Eq 1 for Each Lipid Extracted from Dried *Chlorella* by FAME^{solv}/Methanol Extractant

FAME:methanol	0:100	20:80	40:60	60:40	80:20	100:0	60:40	60:40	60:40
algae(g)/FAME^{solv} (3.2 mL)	1	1	1	1	1	1	5	1	1
extraction time	12 h	12 h	12 h	12 h	12 h	12 h	12 h	3 h	5 h
C16:1	N.D.[a]	N.D.	N.D.	N.D.	5	N.D.	3	N.D.	N.D.
C16:0	10	80	86	127	75	N.D.	86	98	119
C18:2	3	54	56	112	23	N.D.	80	93	105
C18:1	5	94	98	152	91	91	121	121	132
C18:0 (%)	4	12	18	48	44	34	6	42	47
overall REE (%)	5	61	64	106	54	28	78	85	97

[a]N.D.: not detected.

and C18:0 (7%). Because FAME^{solv} is made of rapeseed oil, it has similar components, including C18:1 (36%), C18:2 (27%), C16:0 (21%), and C18:0 (7%). Therefore, it is difficult to analyze FAME^{product} that was prepared from the FAME^{solv}/methanol solvent because FAME^{product} from microalgae coexists with FAME^{solv}. Consequently, equivalent amounts of a standard, heptanoic acid, were added to FAME^{solv} to normalize the GC-MS results.

FAME/Methanol Solvent Extraction Method. To examine the extraction efficiency of FAME^{solv} to the chloroform/methanol, dried algae were mixed with the FAME^{solv}/methanol solvent (80:20, v/v) and stirred for extraction. The purified extracts underwent a transesterification reaction as described above. From the GC-MS analysis, the REE of each lipid against the chloroform/methanol method could be calculated. In dried *Nannochloropsis*, the extraction by FAME^{solv}/methanol was inefficient for all lipids (Supporting Information 6). The most efficiently extractable component (C18:2) was recovered less than 37% compared with the chloroform/methanol method. This is because the dried *Nannochloropsis* was not well dispersible in FAME^{solv}/methanol, and it formed a mass of powder, which may reduce the interaction of the lipids with solvent.[2] However, dried *Chlorella* powder was well dispersible in FAME^{solv}/methanol, and the overall REE was relatively high at 53%. The C18:1, which comprises the largest fraction of *Chlorella* oil, displayed the highest extraction efficiency up to 91%. Furthermore, the recovery efficiency of FAME^{product} along with FAME^{solv} was 97%. It supports that FAME^{solv} is recoverable and recyclable, and a loss of FAME^{solv} is negligible.

To optimize the FAME^{solv}/methanol ratio for efficient extraction, diverse FAME^{solv}-to-methanol ratios were tested for oil extraction from dried *Chlorella*. As listed in Table 1, pure methanol was insufficient to extract the neutral algal oils, and the overall REE was less than 4%. However, addition of FAME^{solv} to the solvent significantly improved the REE. Particularly, the REEs of C18:1, C18:2, and C16:0 were higher than other components, and these components are consistent with the main constituents of FAME^{solv}. This result implies that similar lipid structures with the main components of FAME^{solv} can be efficiently extracted in a selective manner. Specifically, the extraction solvent at a 60:40 ratio of FAME^{solv}/methanol was the most effective, and C18:1 could be extracted 1.5 times more than the chloroform/methanol method. In addition, the overall REE was 106%. The results demonstrate that FAME^{solv} can efficiently extract lipids from important diesel sources, such as C18:1, C18:2, and C16:0, in a selective manner, and the extraction efficiency was comparable to the chloroform/methanol extraction method. Even though a 1:2.81 algae/solvent mass ratio was used in the FAME^{solv}-based extraction method, the REE result is also comparable to the hexane extraction method that uses an algae/solvent ratio of 1:20. (Supporting Information 8)

To verify the effect of algae weight per solvent on the extraction efficiency, we increased the amount of dried *Chlorella* from 1 to 5 g, and FAME^{solv}:methanol (60:40) was maintained at 3.2 mL. The mixed slurry was too thick to process, and it resulted in a reduction in the overall REE (78%) (Table 1). To examine the amount of time required to complete extractions, the extraction time-dependent REE of each lipid component was analyzed, and 97% of REE could be achieved in 5 h (Table 1, Figure 6S, Supporting Information).

Penetration of FAME into Wet Microalgae. The dewatering and drying processes of microalgae are energy intensive, and a wet extraction method without those steps is a worthwhile subject to investigate.[15,16] The water surrounding the cells inhibits the penetration of organic solvents through the cell membrane, and it diminishes the extraction efficiency.[2] Consequently, the extraction solvent should be able to penetrate through the cell membrane at the wet condition in the aqueous phase. The intracellular delivery of FAME^{solv} in freshly cultivated *Chlorella vulgaris* was assessed by tracking the fluorescence of Nile red, which was dissolved in FAME^{solv} (see Supporting Information 2 for detailed experimental procedures).[17] The wet *Chlorella* was used directly after cultivation without freezing or drying. As shown in Figure 3b, confocal laser scanning microscopy analysis showed that FAME^{solv} can penetrate into microalgae in water and can stain lipids in the cells. Notably, the fluorescence intensity of Nile red in cells delivered by FAME^{solv} was higher than that of methanol, and some cells stained by Nile red in methanol showed low intensity. On the basis of the fact that the fluorescence intensity of Nile red increases with oil content, the result indicates that the delivered FAME^{solv} increased the oil content in cells.[17] To prove this assumption, wet *Chlorella vulgaris* was treated with FAME^{solv} by incubation in an aqueous solution mixed with various amounts of FAME^{solv}, and the fluorescence intensity of the wet *Chlorella vulgaris* stained by Nile red in methanol was measured by luminescence spectroscopy. As shown in Figure 3c, the fluorescence intensity increased with the amount of treated FAME^{solv}. It demonstrates efficient access of FAME^{solv} to the oils in wet algae, and it supports the fact that FAME^{solv} can be potentially applicable to the wet extraction.

CONCLUSIONS

We demonstrated that the fatty acid methyl esters of bio-oil can extract lipids from microalgae, and the extraction efficiency is comparable to the chloroform/methanol extraction method.

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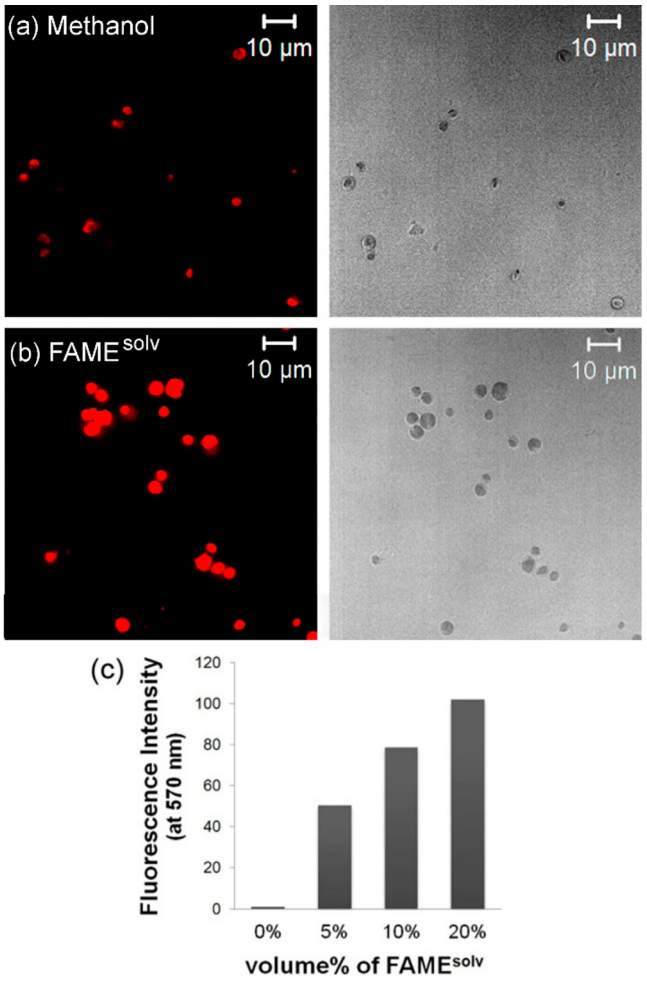

Figure 3. Fluorescence images (left) and differential interference contrast images (right) of Nile red stained wet *Chlorella vulgaris* by (a) methanol and (b) FAME^{solv} observed by confocal laser scanning microscopy. (c) Fluorescence intensity of wet *Chlorella vulgaris* incubated in FAME^{solv}-containing water followed by staining using Nile red dissolved in methanol.

Rapeseed oil-based FAME^{solv} can selectively extract valuable lipids for biodiesel production, such as C18 and C16, because of similar long alkyl chain structures with the main components of FAME^{solv}. Furthermore, FAME^{solv} displayed successful translocation from water to the intracellular environment by cell membrane penetration in an aqueous environment, and this result implies that FAME^{solv} has potential for wet extraction. From this study, we showed that the proposed cycling process, which utilizes a small amount of produced biodiesel as a solvent for the additional oil extraction from fresh microalgae, has a realistic possibility for dried or wet algae extraction. The efficient FAME^{solv}-based extraction process, which does not require toxic solvents, an energy-intensive solvent recovery, or lipid separation processes, provides a new approach for the efficient and effective biodiesel production process in practical and industrial aspects.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials, experimental details, viscosity analysis, GC-MS spectra, extraction results for *Chlorella* and *Nannochloropsis*, mass balance analysis, and time-dependent extraction efficiency. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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