

Article

Energy-Saving Lipid Extraction from Wet *Euglena gracilis* by the Low-Boiling-Point Solvent Dimethyl Ether

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Abstract: We tested a wet extraction method for lipid extraction from *Euglena gracilis* water slurry at 0.51 MPa and 20 °C using liquefied dimethyl ether (DME). The yields, proximate analyses, elemental composition, and molecular weight distribution properties of the extracts from *E. gracilis* and the remaining residues obtained by DME extraction were compared with those of the extracts obtained by hexane Soxhlet extraction.

Keywords: dimethyl ether; *Euglena gracilis*; lipid extraction

1. Introduction

Microalgae have received significant attention as a next-generation bio-fuel resource. Compared with other energy crops, microalgae have higher photosynthetic efficiency, higher biomass production, and a faster growth rate [1]. However, being an aquatic organism, microalgae have a high water content, which causes considerable energy loss during bio-fuel production. The common procedures for algae bio-fuel production include cultivation, harvesting, dewatering, drying, oil recovery, and bio-fuel refining [2–4]. After removing water, normally cell disruption is performed to release products

contained in the microalgae [5]. Recently, Lardon *et al.* conducted a life cycle assessment of algal fuel production, and indicated that drying and solvent evaporation steps consumed huge amounts of energy, resulting in a negative energy balance for algal fuel production [6,7]. Generally, algal lipids are extracted by supercritical CO₂ and organic solvents such as hexane [8]. Gaseous carbon dioxide changes into the supercritical state above its critical point (T_c : 31.1 °C, P_c : 7.3 MPa), and exhibits liquid-like density and gas-like viscosity [9]. Supercritical CO₂ is the most used supercritical fluid, because it is nonflammable, harmless, and inexpensive compared with other chemicals. Supercritical CO₂ has a high selectivity for fractionating the high-quality components by temperature and pressure control [10]. However, drying the microalgae is a necessary pretreatment step for supercritical CO₂ extraction because supercritical CO₂ is a nonpolar solvent and its solubility in water is very low [11]. Moreover, special apparatuses are required to withstand the high pressure of supercritical CO₂ [12]. Furthermore, lipid solubility in supercritical CO₂ is lower than that in organic solvents such as hexane, because the density of supercritical CO₂ is lower than that of organic solvents [13].

In a previous study, we proposed using liquefied dimethyl ether (DME) as a low-boiling-point solvent for extraction because DME can reduce the energy required for drying (dewatering) and solvent evaporation [14–16]. In addition, DME has been developed as a synthetic fuel for use in both liquid and gaseous forms. In China, DME is synthesized using small-scale coalfields of low commercial value and produced as a fuel at a cost equivalent to that of imported liquefied petroleum gas [17–19]. The boiling point of DME is −24.8 °C and its saturated vapor pressure at 20 °C is 0.51 MPa [20]. As a hydrogen bond acceptor, DME forms weak hydrogen bonds, and only 7–8 wt.% water dissolves in liquefied DME at ordinary temperature. Since the quantity of DME gas that dissolves in water is low, it can be easily separated from water by simple flash distillation at ordinary temperature [21]. Furthermore, liquefied DME has been examined as a prospective solvent in food processing because of its low toxicity [22,23]. DME differs from typical ethers such as ethyl ether since it does not form peroxides [24]. Previously, lipids were successfully extracted from seven wet cyanobacteria microalgae samples by DME, thus eliminating the need for cell drying and cell disruption and the use of a high-temperature heat source for solvent evaporation [14]. In addition to cyanobacteria, other forms of microalgae such as green algae and diatoms exist. While green algae and diatoms are eukaryotes, cyanobacteria are prokaryotes. The cell wall of cyanobacteria is mainly composed of peptidoglycan, whereas the main component of the cell wall of some green algae is cellulose and that of diatoms is silica. Since the components of the microalgae cell are different, the findings of the previous study using cyanobacteria may not be applicable to green algae or diatoms.

In this study, we tested DME extraction on wet *E. gracilis*, which has a simple cell structure and the ability to produce diverse valuable organic components, including lipids [25,26]. The extraction efficiency and extract properties were compared to those of the classical lipid extraction method using Soxhlet extraction with hexane.

2. Results and Discussion

2.1. Extraction Yield, Elemental Analysis, and Higher Heating Value of the Extract

These analyses were repeated three times for each extraction. The results show the average value. Statistical analysis of the data was performed by the ANOVA analysis and the Bonferoni test ($p < 0.05$ and $p < 0.01$) in order to compare different data generated in the experiments. These analyses were repeated three times for each extraction. The results show the average value. As shown in Figure 1, the best final extraction yield of 32.5% (dry basis) was achieved by passing 359.5 g of liquefied DME through the extractor. Furthermore, water was simultaneously removed from wet samples and the dewatering ratios are shown in Figure 1. The lipid extracted by liquefied DME was easily separated from water by a simple oil-water separation technique after the evaporation of liquefied DME. In contrast, the extraction yield obtained by hexane Soxhlet was 31.8%. Therefore, the efficiency of the DME extraction compared to the hexane Soxhlet was 102.4%, which demonstrates that an accurate extraction ratio, similar to that of the hexane Soxhlet, can be achieved using liquefied DME.

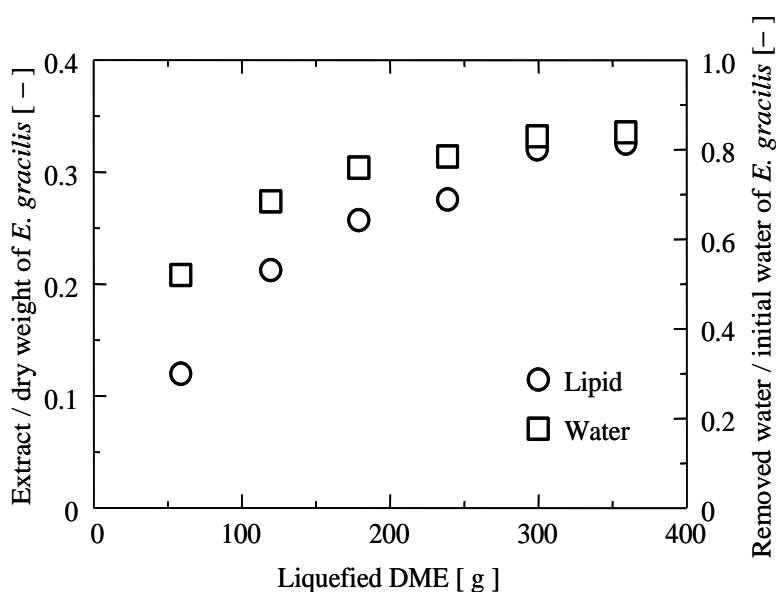


Figure 1. Extraction yields from wet *E. gracilis* with liquefied DME.

The upper half of Table 1 shows the proximate and higher heating value (HHV) of *E. gracilis* and the extracted residues and lipids. First, the ash content of *E. gracilis* was 6.2%. The ash contents of the residue and lipid by liquefied DME extraction were 8.2% and 1.2%, respectively, while those by hexane were 8.1% and 1.4%, respectively. The ash concentrations in the lipids obtained by liquefied DME and hexane Soxhlet were 1.2% and 1.4%, respectively, which are undesirably high and require the removal of the ash using additional refining.

The volatile matter and fixed carbon content of *E. gracilis* were 83.2% and 10.6%, respectively (Table 1); the residue by liquefied DME were 75.2% and 16.6%, respectively; and those of the residue determined by hexane were 76.2% and 15.7%, respectively. Therefore, both the liquefied DME and hexane Soxhlet extraction gave slightly different results from those of *E. gracilis* because the volatile lipid was removed.

Table 1. Proximate analysis, higher heating value, and elemental analysis.

Analysis	<i>E. gracilis</i>	Liquefied DME		Hexane	
		Residue	Lipid	Residue	Lipid
Proximate analysis (wt.% dry basis)					
Ash yield	6.2	8.2	1.2	8.1	1.4
Volatile matter	83.2	75.2	-	76.2	-
Fixed carbon	10.6	16.6	-	15.7	-
Higher heating value (MJ/kg)	34.5	29.3	40.8	29.5	40.7
Main elements (wt.% dry basis)					
C	55.5	45.2	76.5	46.9	76.3
H	8.7	6.4	12.4	6.6	12.3
N	6.8	9.8	2.4	9.5	2.2
O *	21.9	29.2	7.4	27.7	7.7
S	0.87	1.23	0.02	1.21	0.01
(Non-combustible S)	<0.01	<0.01	-	<0.01	-
Minor elements (ppm)					
Na	-	-	320	-	30
K	-	-	4500	-	680
Mg	-	-	1900	-	250
Ca	-	-	150	-	170
Al	-	-	3	-	<1
Zn	-	-	34	-	10
Fe	-	-	38	-	43
P	-	-	6000	-	1400

Note: * By calculation.

The HHV of *E. gracilis* was 34.5 MJ kg⁻¹, while the corresponding values of the residue and lipid by liquefied DME were 29.3 and 40.8 MJ kg⁻¹, respectively, and those by hexane were 29.5 and 40.7 MJ kg⁻¹, respectively. For both the liquefied DME and hexane extractions, the HHVs of lipids were higher than that of *E. gracilis*.

The elemental composition of *E. gracilis*, residues, and lipids are shown in the lower half of Table 1. The lipids extracted using liquefied DME consisted mainly of C, H, N, and O (76.5%, 12.4%, 2.4% and 7.4%, respectively). The C and H contents of the lipids are higher than those of the raw *E. gracilis*; however, the N and O contents are much lower. In particular, the S concentrations of the extracts by both the methods are 0.02%, which are significantly lower than those of the raw *E. gracilis*.

As described, the ash contents, volatile matter contents, fixed carbon contents, HHVs, and main element amounts of the residues and lipids by liquefied DME are almost the same as those by hexane, despite the omission of the drying and cell disruption steps in the liquefied DME extraction.

In the liquefied DME extraction, the concentrations of group IA elements Na and K in the lipid were 320 and 4500 ppm, respectively (Table 1), and the concentrations of group IIA elements Mg and Ca were 1900 and 150 ppm, respectively. The P concentration is also an important factor because very low levels of P may lead to unexpected engine deterioration [27]. The concentration of P that remained in the extracted lipids was 6000 ppm. This result implies that phosphatide is efficiently extracted by liquefied DME. As a reference value, the European Standard EN 14214 limits are <5.0 ppm for both

group IA elements (Na and K) and group IIA elements (Ca and Mg), and <4 ppm for P [28]. The concentrations of Al, Zn, and Fe were 3, 34 and 38 ppm, respectively. These concentrations obtained by liquefied DME are higher than those obtained by hexane Soxhlet because liquefied DME is a more polar solvent and it is capable of extracting the nutrient medium. The analytical results presented above indicate that a subsequent refining process is required for further purification.

2.2. Molecular Weight Distribution

Figure 2 shows molecular weight distribution curves of the extracts determined relative to polystyrene standards. The molecular weight distribution curves obtained by liquefied DME were compared with those obtained by hexane Soxhlet extraction using gel permeation chromatography analyses using both chloroform and tetrahydrofuran (THF). Using chloroform, the average molecular weights (M_w) and the number average molecular weights (M_n) of the nonpolar extracts were 910 and 820 g mol^{-1} by DME and 910 and 810 g mol^{-1} by hexane Soxhlet extraction, respectively. Using THF, the M_w and M_n values of the polar lipids were 820 and 720 g mol^{-1} for the DME extraction and 830 and 720 g mol^{-1} for hexane Soxhlet, respectively. The molecular weight distributions of both the nonpolar and polar lipids obtained by DME are almost the same as those obtained by hexane Soxhlet. This similarity is due to the soft cell structure of *E. gracilis*, because it does not have a cell wall. Therefore, all nonpolar and polar components were extracted by both solvents (liquefied DME and hexane); thus, the molecular weight distributions are independent of the solvent polarity.

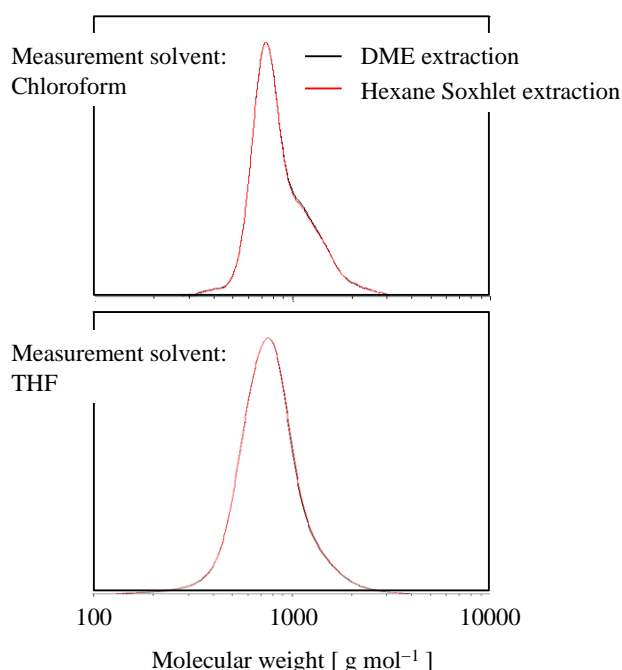


Figure 2. Molecular weight distributions of the extracts.

2.3. Procedure Comparison of Proposed and Conventional Methods

Recent studies of algae bio-fuel have found that the combination of hexane and a traditional extraction method such as a Soxhlet are an efficient and feasible method for the recovery of lipids from microalgae [29]. As a non-polar solvent, hexane has very good selectivity for cellular non-polar or

neutral lipids. However, because hexane is a hydrophobic solvent, it is not able to penetrate wet algal cells to extract lipids from inside the cells. Therefore, pretreatment processes such as dewatering and drying are necessary. Several recent attempts were made to use hydrophilic organic solvents or supercritical fluid technology conceptualized as a wet extraction. However, until now none of the methods identified were both practical and economical. The procedures for the proposed DME extraction and classical hexane extraction are shown in Figure 3. In comparison to the conventional method, drying and solvent evaporation at high temperatures are not required for the DME extraction. The operational temperature shown in Figure 4 can be set arbitrarily close to the ordinary temperature and that shown as 30 °C in the figure is a typical example. The operational pressure is the saturated vapor pressure of DME. The extraction column was packed with wet microalgae collected after mechanical dewatering. The typical water content of the collected microalgae was about 90 wt% [14]. Water and lipids were extracted from the wet microalgae by liquefied DME, and the liquefied DME was discharged from the extraction column as a mixture with lipids and water. In order to increase the concentration of lipids in the liquefied DME, the liquefied DME was returned to the extraction column until the lipid fraction was suitably concentrated. The lipid containing liquefied DME was then heated to evaporate the DME at 30 °C in a heat exchanger by a hot heat source. As the hot heat source, sun-warmed water or waste heat generated by a small biomass electric power plant, which have commonly low electricity generation efficiency, are desirable. The 45 °C setting of hot water shown in the figure is just an example, and it is advisable to increase the approach temperature in order to reduce the size of the heat exchanger. The evaporated DME gas at elevated temperatures and high pressures can then be condensed using a cooling source such as seawater in order to reuse the liquefied DME. The temperature of 15 °C shown for the cold heat source is just an example. The size of the heat exchanger was reduced by lowering the temperature of the cold source such as seawater. Ultimately, the temperature of the circulated DME can be determined based on the temperature of the cold heat source and the approach temperature of the heat exchanger.

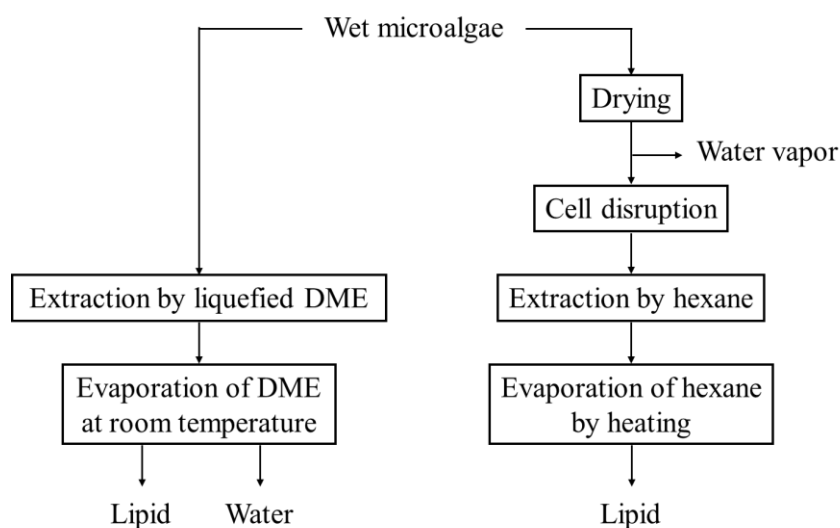


Figure 3. Comparison of the liquefied DME and typical hexane extraction procedures.

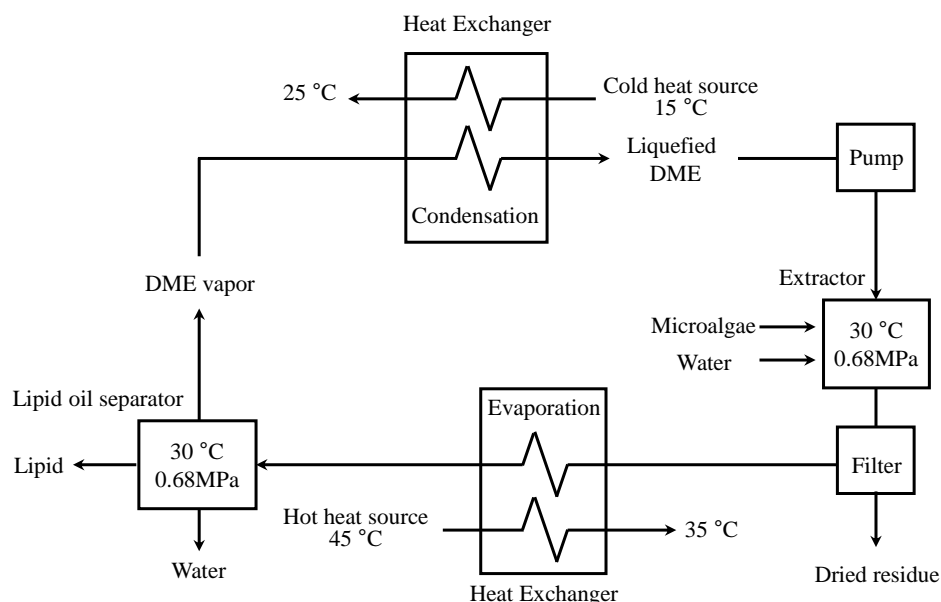


Figure 4. Process concept of energy-saving extraction by liquefied DME.

Energy consumption of the liquefied DME-sending pump can be estimated using the official basic specs of the pump, the quantity of solvent required to extract the lipids, and the amount of lipid extracted. Commercially available liquefied petroleum gas-sending pumps can be used as the liquefied DME-sending pump. The leak-proof seal of the sending pump should be coated with Teflon, which offers superior resistance to DME corrosion [30]. As an example, the official basic specs of a large-scale commercially available LPG-sending pump (Model number; E-516F2M-0810U1-E/F, Tokyo Boeki Mechanics Ltd., Tokyo, Japan) are as follows: flow rate, 800 L min^{−1}, or 13.33 L s^{−1}; power requirement, 15 kJ s^{−1}; and receiving end efficiency (which is the ratio of electric energy received by the pump based on the HHV of fossil fuel consumed in a thermal power plant) in Japan, 0.369. Therefore, 0.570 g (extracted lipid = 32.5% of dry weight of *E. gracilis*, water content of *E. gracilis* paste = 80.3%) of lipids obtained were extracted by 0.544 L of liquefied DME (359.5 g, density of liquefied DME at 20 °C is 0.661 g cm^{−3}).

Here, the energy consumption (E) of the liquefied DME-sending pump per kg of lipid is calculated by the following equation:

$$E = V/L \times W/F/R = 2.96 \text{ MJ kg}^{-1}$$

where V is the volume of the liquefied DME (0.554 L); L is the weight of lipids extracted by liquefied DME V (0.570 g), W is the energy requirement (15 kJ s^{−1}); F is the flow rate of liquefied DME (13.33 L s^{−1}); and R is the receiving end efficiency in Japan (0.369). Moreover, if counter flow extraction by a simulated moving bed could be employed for the DME extraction technique, the ratio of V/L would be smaller than that obtained in this experiment. Therefore, energy consumption of the liquefied DME-sending pump would be much smaller than 2.96 MJ kg^{−1}. This implies that the energy consumption of the liquefied DME-sending pump is clearly smaller than the HHV of the extracted lipid.

3. Experimental Section

3.1. DME and Hexane Soxhlet Extraction

E. gracilis was provided by JX Nippon Oil & Energy Corporation, Tokyo, Japan. It was grown in a modified Cramer-Myers medium maintained at 29 ± 3 °C. The pH was maintained in the range 2–4.5 in order to prevent contamination through the supply of CO₂. The depth of the culture solution was maintained at about 0.30 m to maintain efficient photosynthesis. For extraction test pretreatment, the *E. gracilis* cells were roughly dewatered by suction filtration. The water content of the *E. gracilis* paste was 80.3%, as determined by continual drying at 107 °C to constant weight. Wet *E. gracilis* was then used in the DME extraction. A liquefied DME supplier (volume: 100 cm³; TVS-1-100, Taiatsu Techno Corp., Saitama, Japan), an extraction column (inner diameter: 11.6 mm, HPG-10-5, Taiatsu Techno Corp.), and a storage tank for the extract (HPG-96-3, Taiatsu Techno Corp.) were connected in series, as shown in Figure 5. Based on an average of three tests, 8.91 g of wet *E. gracilis* was placed in the lower half of an extractor, and glass beads (0.71–0.99 mm diameter) were placed in the upper half of the extractor. The extractor was pressure-resistant glass coated with polycarbonate, and was positioned in a water tank to maintain the temperature at 20 °C. Liquefied DME was supplied to the extractor by using 0.7 MPa of nitrogen gas and the flow rate was adjusted by a pressure depression valve. At a predetermined temperature and pressure, liquefied DME was passed through the extractor at a flow rate of 10 ± 1 cm³ min⁻¹.

For the Soxhlet extraction with hexane, heat-dried *E. gracilis* were disrupted for 5 min at 10,000 rpm in hexane using a homogenizer equipped with a 10-mm outside diameter sawtooth generator probe (Dremel 300 Series; Robert Bosch Tool Corp., Mount Prospect, IL, USA). The time and temperature employed for the hexane Soxhlet extraction were 18 h and 69 °C, respectively.

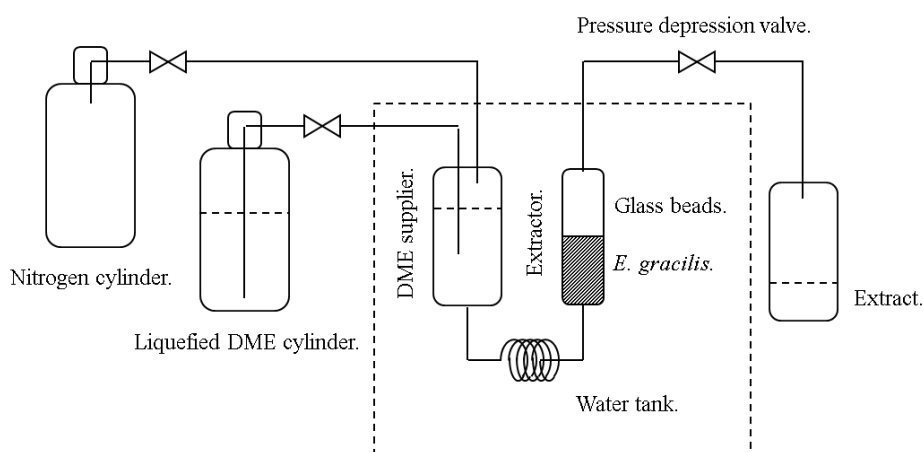


Figure 5. Schematic diagram of the DME extraction apparatus.

3.2. Compositional Analyses

The ash contents, higher heating value, and main elements amounts were analyzed for raw *E. gracilis*, residues, and lipids obtained by liquefied DME and hexane extractions.

Volatile matter and fixed carbon contents, which are typically analyzed for coal, were analyzed for the original *E. gracilis* and *E. gracilis* residue. Minor elemental contents, which are typically analyzed

for crude oil, were analyzed only for lipids. *E. gracilis*, residues, and lipids were pretreated for these analyses by continually drying at 107 °C to constant weight.

The ash contents were determined according to the analytical method of the Japanese Industrial Standard (JIS) Z 7302-4:1999, the volatile matter contents and fixed carbon contents were determined according to the analytical method of JIS M 8812:2004 7, and the higher heating values were determined according to the analytical method of JIS Z 7302-2:1999.

The carbon, hydrogen, and nitrogen contents were determined according to the analytical method of JIS Z 7302-8:2002. The oxygen contents were calculated by difference. The sulfur content was determined according to the analytical method of JIS Z 7302-7:2002 7.1b.

The concentrations of minor elements such as Na, K, Ca, Mg, Ca, Al, Zn, and Fe were determined by atomic absorption spectrometry (Z-2000; Hitachi High-Technologies Corp., Tokyo, Japan) according to the Japanese industrial standard method of JIS K 0102:2008 48.2, JIS K 0102:2008 49.2, JIS K 0102:2008 50.2, and JIS K 0102:2008 51.2, which correspond to ISO 14911:1998. Phosphorus concentrations were determined by potassium peroxodisulfate digestion molybdenum blue absorption spectroscopy (U-2001; Hitachi High-Technologies Corp.) according to the analytical methods of JIS K 0102:2008 46.3.2, which corresponds to ISO 6878:2004.

The molecular weight distributions of the lipids obtained by liquefied DME and hexane were determined by gel permeation chromatography performed at 40 °C by diluting the extracts in either chloroform or THF. Chloroform is a suitable solvent for the nonpolar components of the lipids. THF is a suitable solvent for polar components. For chloroform, one Shodex K-800D and two K-805L columns (Showa Denko K.K., Tokyo, Japan) were employed. For THF, one Shodex KF-G, two KF-805L, and KF-800D columns (Showa Denko K.K.) were used. These properties were compared with the results of the hexane Soxhlet.

4. Conclusions

We have demonstrated that liquefied DME extraction is suitable for wet *E. gracilis*. The lipid extraction yield, proximate analysis results, C, H, N, O, and S compositions, and molecular weight distribution of the lipid obtained by liquefied DME were identical to those obtained by hexane. In contrast to conventional Soxhlet extraction, this method does not require drying, cell disruption, or solvent evaporation at high temperatures. Minor element concentrations in the lipid by liquefied DME were higher than those obtained by hexane because of weak hydrogen bonds of liquefied DME, which indicates another refining process is required for further purification.

Acknowledgments

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Author Contributions

Hideki Kanda contributed to the overall idea, planning, financing, and writing of the manuscript. Peng Li contributed to the experiment, analysis, interpretation, and writing of the manuscript. The research direction was provided by Motonobu Goto and Hisao Makino.

Conflicts of Interest

The authors declare no conflict of interest.

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