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Method Article

Microalgae preparation and lipid extraction by subcritical dimethyl ether

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A B S T R A C T

Biodiesel produced from microalgae is a potential alternative due to the high growth rate of microalgae, the possibility of using nonarable land, and high lipid accumulation rate. Microalgae cultivation, cell harvesting and disruption are the important steps before lipid extraction for the biodiesel. In the co-submission article, the details of the whole process cannot be clearly explained. In this regard, we present the details of methods on parameter of photo-bioreactor for cultivating microalgae, flocculation tests to determine optimal flocculant dosage in harvesting, parameter of Dimethyl ether (DME) subcritical extraction device and full-factorial design for investigating the influence of extraction time, initial water content and DME dosage on the extraction performance. It will allow researchers to reproduce these experiments.

- The method shows a cell disruption assisted lipid extraction by subcritical dimethyl ether.
- Model is built from full-factorial design to investigate multi-factor influence.
- Differential scanning calorimetry can be applicable to measure free water content.

A R T I C L E I N F O

Method name: Method to prepare microalgae and lipid extraction
Keywords: Microalgae cultivation, Biomass harvesting, Subcritical extraction, Water content
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Specifications Table

Subject Area	Energy
More specific subject area	*Biomass for lipid extraction*
Method name	*Method to prepare microalgae and lipid extraction*
Name and reference of original method	*See the main article (co-submission).*
Resource availability	*All resource information needed to reproduce this method is integrated in the paper. (i.e. reagent names, equipment, software).*

Microalgal cultivation

As shown in the Image 1, the device for cultivating consists of 5 parts: metal frame, illumination, bioreactor, aeration system and power control. The metal frame is utilized to fix illumination. The illumination is realized by two rows of fluorescent lamps, which can supply a full spectrum to simulate sunlight. Bioreactors are square buckets of transparent material with a lid with two small holes. One hole is for the aeration input, and another hole is for air outlet. Aeration system provides air through an air pump. The pumped air is washed with ultrapure water and then passed through the filter to remove fine particles and organisms. The filtered air enters the bioreactor through the aeration head placed at the bottom of the bucket. The power control simulates the alternation of day and night by setting the timing to turn on the lights.

The freshwater microalgal species *T. obliquus* was obtained from the Microbial Culture Collection at the National Institute for Environmental Studies (Tsukuba, Japan). The microalgae were cultivated in the 20-L square bucket photobioreactor (320 × 229 × 399 mm, Polycarbonate) under a 12 h/12 h light/dark cycle by the AF-6 growth medium [1]. The bucket was placed between two rows of fluorescent lamps (40 W, full spectrum). The distance between the lamp tubes and the bucket wall

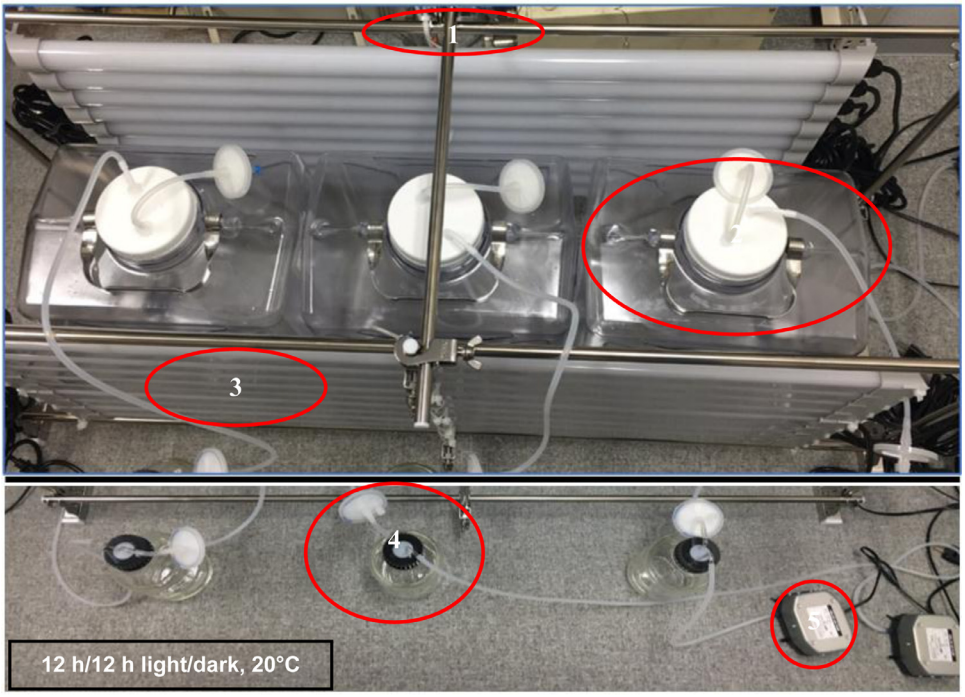

Image 1. Photo-bioreactor for cultivating microalgae.
*1 metal frame; 2 bioreactors; 3 illumination; 4 air filtration devices; 5 air pump.

was 15 cm. During cultivation, three lamps on each row were opened, the temperature was controlled at 20°C. And filtered air was continuously aerated into the medium through aeration head with an effect of agitation. The biomass concentration was monitored by spectrophotometry (UV-1200; Shimadzu, Kyoto, Japan) according to optical absorbance (OD) at 680 nm every day. Cultivation was complete, once the OD stopped increasing and the microalgae entered the stationary phase (after ~20 days). The dry cell weight of microalgae in the stationary phase was measured by filtration using pre-weighed glass microfiber filter paper (GF/C; 1.2 μm; 4.7 cm; Whatman plc, Maidstone, UK), which was then dried in an oven (105 °C for 24 h) and re-weighed. This process was repeated in triplicate; the obtained dry cell weight was 1.31 ± 0.01 g/L.

Microalgal harvesting and sample preparation

(a) To evaluate the effect of water in microalgae on the DME extraction performance, the cultivated microalgae were first harvested by centrifugation (5000 rpm, 10 min, Beckman Coulter Avanti J-26S XP, JLA-10.500 6 rotors), and then dried in an oven at 105 °C. The dried samples were ground in a mortar and pestle to a powder, which was stored in Super-Dry (SD-302–01, Tokyo Garasu Kikai Co., Ltd., Tokyo, Japan). Before lipid extraction, this powder was mixed with ultrapure water (Wako Co., Ltd., Tokyo, Japan) to prepare microalgal samples with specific water contents. Specifically, 7:13, 1:3 and 3:17 (w/w) of microalgal powder and ultrapure water were evenly mixed in tube to obtain samples with water content of 65%, 75% and 85% respectively. The prepared samples were immediately subjected to the lipid extraction experiment.

(b) To evaluate cell disruption, the cultivated microalgae were flocculated by $AlCl_3$ or chitosan. A pre-experiment was conducted to determine optimal flocculant dosage (for maximum microalgae harvesting efficiency). The flocculation experiments were performed in 100 mL beakers. After adding flocculant by pipette, the microalgae suspensions (in the stationary phase, dry cell weight of 1.31 g/L) were intensively mixed (350 rpm) for 2 min, followed by a gentle mixing (50 rpm) for 10 min by magnetic stirring (MG120; Yamato Scientific Co., Ltd., Tokyo, Japan). The suspensions were subsequently allowed to settle for 15 min. The supernatant was sampled from the middle of the clarified zone and the OD was measured. The harvesting efficiency was calculated by Eq. (1) as follows:

$$\text{Harvesting efficiency } (\%) = \left(1 - \frac{OD_{final}}{OD_{initial}}\right) \times 100 \quad (1)$$

where $OD_{initial}$ was the optical absorbance before flocculation, and OD_{final} was the optical absorbance after flocculation was completed.

By the pre-experiment, the optimal flocculant dosage for $AlCl_3$ or chitosan was determined to be 45 mg/L (harvesting efficiency of ~ 96%) and 40 mg/L (harvesting efficiency of ~ 98%) respectively. Thus, the microalgal floc was collected by adding the optimal dosage of flocculant with the same process described above but in a 2 L bucket, while supernatant was carefully removed with a peristaltic pump and pipette.

The five cell disruption methods (Section 3) were applied to the collected microalgae floc. After cell disruption, these samples were dewatered by filtration. The blank samples were prepared with $AlCl_3$ + filtration, $AlCl_3$ + centrifugation, chitosan + filtration, and chitosan + centrifugation or just centrifugation, without any cell disruption treatment. The details of these processes with their abbreviation were shown in the Table 1.

The microalgae are firstly flocculated by $AlCl_3$ or chitosan for reducing volume into downstream treatment. Then the flocs are conducted to various cell disruptions. Finally, filtration of these treated flocs can reduce water content of samples for lipid extraction. The blank group A (AF and CF; flocculation-filtration) contrasts the effect of cell disruptions, blank group B (AC and CC; flocculation-centrifugation) and C (OC; only centrifugation) is used to eliminate the influence from dewatering types and flocculants.

Table 1
Microalgae samples preparation and their abbreviation.

		OC	AC	CC	AF	CF	AAF	CAF	AGF	CGF	AHF	CHF	AMF	CMF	AUF	CUF
Flocculation	$AlCl_3$		√		√		√		√		√		√		√	
	Chitosan			√		√		√		√		√		√		√
Cell Disruption	Acidification						√	√								
	Homogenization								√	√						
	Heating										√	√				
	Microwave												√	√		
	Ultrasonic														√	√
Dewatering	Centrifugation	√	√	√												
	Filtration				√	√	√	√	√	√	√	√	√	√	√	√

* One missing letter "F" in the abbreviation means the sample has not been filtered yet. Such as CA means the sample was flocculated by $AlCl_3$ and then treated by acidification. An additional letter "D" in the abbreviation means that the sample has been lipid-extracted by DME. Such as CAFD means the sample was flocculated by chitosan, treated by acidification, filtered and then mixed with DME for lipid extraction Sequentially.

Cell disruption treatments

Microalgal samples (each of 30 mL) obtained by flocculation with $AlCl_3$ or chitosan were treated by the following five methods respectively. (1) Microwave irradiation, a microwave digestion system (2450 MHz, ETHOS One, Milestone Inc., USA) equipped with ten 100 mL Teflon reaction vessels was used. After the microalgal samples were placed in the reaction vessels, they were sealed and heated via microwave irradiation for approximately 1 min to achieve a holding temperature of 85°C. The samples were maintained at the holding temperature for 30 min. The output of the microwave oven was set at 500 W during the heating and holding process. (2) Water bath heating, the microalgal samples were placed in centrifugation tubes (50 mL) and heated in a water bath at 85°C for 30 min. (3) Acidification, the HCl (37.5%) was added into centrifugation tubes with the microalgal samples to adjust the pH to 1.0, and waiting 2 h at room temperature. (4) Homogenization, the samples were placed in the centrifugation tubes and put homogenizer (10,000 rpm) in the center of liquid with an immersion depth of 3/4 below the liquid level for 10 min. (5) Ultrasonication, an ultrasonic processor (UD-211; Tomy Digital Biology Co., Ltd., Tokyo, Japan) with a Titanium alloy Horn Tip (14.5 mm diameter x 3 mm, Flat shape) was used. The instrument operated at constant ultrasound amplitude, power, frequency and duty cycle of 100 μm, 15 W, 20 kHz, 50%, respectively. The samples were introduced into 60 mL capacity (100 mm height, 30 mm internal diameter) plastic vessels. The tip of the sonicator horn was placed in the center of the samples, with an immersion depth in the liquid of 15 mm. The ultrasound treatments were performed for 30 min.

Dimethyl ether (DME) extraction method

Procedure overview

The device (Fig. 1) used in the DME method was an apparatus comprised of five parts: a vessel to store liquefied DME (vessel 1; TVS-1–100; 500 cm^3; Taiatsu Techno Corp., Saitama, Japan), a vessel to measure DME (vessel 2; 100 cm^3; HPG-96–3, 26.5 mm diameter × 238 mm; Taiatsu), a vessel for lipid extraction (vessel 3; 100 cm^3; HPG-96–3; 26.5 mm diameter × 238 mm, Taiatsu), a vessel for separating liquid from solvents (vessel 4; 100 cm^3; HPG-96–3; 100 cm^3; Taiatsu), and a $CaCl_2$ moisture-trap column (HPG-10–5; 11.6 mm diameter × 190 mm; Taiatsu). The N_2 gas can be used to push DME through the device. The whole system is sealed to maintain a pressure of 0.55 Mpa. During the experiments, harvested microalgal samples were loaded into extraction vessel 3 containing a cellulose thimble (glass fiber; 25 mm diameter × 100 mm; Whatman, Maidstone, UK) inside. Liquefied DME stored in vessel 1 was pushed into vessel 2 and then into the extraction vessel. After several minutes of extraction (0.55 Mpa, 20°C), the liquid containing raw lipids and water in the vessel was transferred to vessel 4. Releasing the pressure caused the DME to evaporate, leaving two phases of raw lipids and water. The evaporated gaseous DME can be liquefied again at the low temperature or

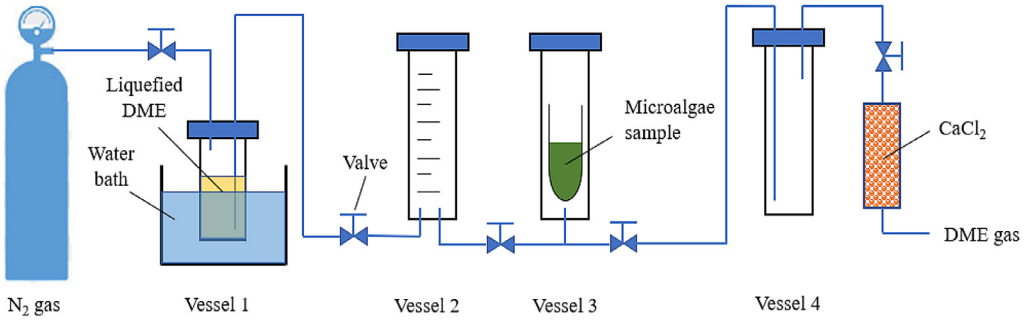

Fig. 1. Schematic of the DME flow-type experimental apparatus.

the high pressure. Theoretically, in a sealed system, the DME recovery rate can be expected to be nearly 100%. Yet, it is difficult to achieve the full recovery of DME by the experimental devices mainly designed for lipid extraction, due to possible remaining in the system. Even so, in our previous study, the DME recovery rate in the devices was measured to be > 90% [2]. In this study, since the reusability of the DME was not focused, the DME was not recycled.

The separated raw lipids were dissolved in 5.0 mL chloroform and filtered through a 0.45 μm membrane filter (DISMIC-13HP; Advantech Co., Ltd., Tokyo, Japan). The solvent was then removed using flowing N2 gas (Nitrogen Thermovap Sample Concentrator; Ishii Tatami Shop Co., Ltd., Kyoto, Japan), and the recovered raw lipids were weighed and stored below 0°C for further analysis.

Experimental design

The samples from 2 (a) were subjected to a full-factorial design (FFD) [3] to mainly examine the effect of water in microalgae on the DME method. Initial water content (65, 75, 85%), extraction time (20, 40, 60 min) and DME dosage (75, 125, 175 mL DME/g D.W. microalgae) were the three factors evaluated at 3 levels with the relative extraction rate and solids content change as the response variable. Twenty-seven experiments repeated three times were thus carried out, and the experimental data were fitted to a second-order polynomial equation (Eq. (2)) by Minitab 18 (State College, PA, USA):

$$Y(\%) = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (2)$$

where Y is the response variable; b_0 is the offset term; b_1, b_2, b_3 are the linear terms influencing the response; b_{11}, b_{22}, b_{33} are the quadratic terms; b_{12}, b_{13}, b_{23} are the interaction terms; X_1, X_2, X_3 are respectively the extraction time, initial water content and DME dosage.

The Table 2 shows a full-factorial design and results to investigate the influence of extraction time, initial water content and DME dosage on Relative raw lipids rate and Solid content. For samples with an initial water content of 85%, the relative extraction rate and residue solids content are 69.2 ± 1.3% and 17.9 ± 1.4%, respectively, when using 75 mL DME/g microalgae (dry basis) and an extraction time of 20 min. These values increase to 75.8 ± 1.9% and 21.0 ± 2.8%, respectively, when the DME dosage is increased to 125 mL/g and the extraction time to 40 min. Using a DME dosage of 175 mL/g and extraction time of 60 min, the relative extraction rate and solids content are 78.1 ± 3.3% and 26.9 ± 1.6%, respectively. Similarly, in the samples with an initial water content of 75%, the relative extraction rate and solids content are 71.9 ± 0.3% and 28.6 ± 2.0%, respectively, at a DME dosage of 75 mL/g and extraction time of 20 min. And they increase to 77.7 ± 1.7% and 38.1 ± 2.4%, respectively, at 125 mL/g DME and 40 min and finally to 84.7 ± 2.9% and 47.2 ± 1.3%, respectively, at 175 mL/g DME and 60 min. In the samples with an initial water content of 65%, the minimum relative extraction rate (76.0 ± 0.3%) and residue solids content (40.8 ± 1.6%) are obtained using 75 mL/g DME and 20 min extraction, while the maximum values (90.7 ± 2.8% and 51.0 ± 2.7%, respectively) are obtained using 175 mL/g DME and 60 min extraction.

Table 2
Experimental and fitted model results for raw lipid extraction and dewatering performances under different water contents.

Extraction time (min)	Initial water content (%)	DME dosage (ml/g)	Relative raw lipids rate (%)		Solid content (%)	
			Experimental value	Predicted value	Experimental value	Predicted value
20	85	75	69.2 ± 1.3	69.9	17.9 ± 1.4	14.9
20	65	175	81.3 ± 4.0	81.0	46.7 ± 0.0	46.6
20	75	125	74.7 ± 0.9	74.0	34.5 ± 1.8	34.7
20	75	175	77.0 ± 1.6	76.8	37.1 ± 1.8	38.3
20	65	125	78.1 ± 0.6	78.2	47.9 ± 1.4	44.5
20	85	125	71.7 ± 1.5	71.8	19.9 ± 0.2	19.3
20	75	75	71.9 ± 0.3	72.0	28.6 ± 2.0	31.8
20	85	175	74.4 ± 1.9	74.4	24.2 ± 1.0	24.4
20	65	75	76.0 ± 0.3	76.1	40.8 ± 1.6	43.1
40	65	175	84.7 ± 0.8	86.0	49.6 ± 1.5	50.1
40	75	125	77.7 ± 1.7	78.2	38.1 ± 2.4	37.9
40	85	75	73.8 ± 3.1	73.2	17.9 ± 0.4	17.7
40	85	125	75.8 ± 1.9	75.8	21.0 ± 2.8	22.7
40	75	175	81.6 ± 3.2	81.6	43.5 ± 2.9	42.0
40	75	75	76.8 ± 1.9	75.6	34.0 ± 0.5	34.4
40	65	125	82.9 ± 0.8	82.6	47.5 ± 1.2	47.6
40	65	75	79.5 ± 3.2	79.8	47.8 ± 2.1	45.6
40	85	175	79.0 ± 2.8	79.1	27.0 ± 1.4	28.3
60	65	175	90.7 ± 2.8	89.8	51.0 ± 2.7	51.9
60	75	125	80.9 ± 2.0	81.2	38.0 ± 1.8	39.4
60	85	75	75.4 ± 1.3	75.3	16.1 ± 0.3	18.7
60	75	175	84.7 ± 2.9	85.2	47.2 ± 1.3	44.0
60	65	125	86.1 ± 2.4	85.7	45.5 ± 2.8	48.8
60	85	125	78.1 ± 3.3	78.5	26.9 ± 1.6	24.3
60	75	75	77.2 ± 1.3	77.9	36.8 ± 1.6	35.3
60	85	175	83.1 ± 4.0	82.5	29.8 ± 3.4	30.4
60	65	75	82.4 ± 0.8	82.4	47.9 ± 8.2	46.4

* The mean values above were taken from the triplicate samples.

Considering the experimental results obtained above and the water content of samples from 2 (b), the condition of experiments for evaluating the cell disruption was DME dosage of 75 mL/g D.W. microalgae and extraction time of 40 min.

Bligh & Dyer method

The B&D method [4] was usually used as a standard for the total lipid content of microalgae. It should be noticed that the B&D method cannot recover whole lipids but most of it in microalgae. The reason for selecting B&D as the benchmark is because of its reliability and commonality. In the Co-Submission, the comparison between the B&D and DME method was reported

In the method, 0.5 g of totally dried microalgae (by oven, 105°C) were mixed in methanol/chloroform/pure water (5.0/2.5/2.0 mL; Guaranteed Reagent, Wako) in a centrifuge tube. The mixture was crushed in a homogenizer (T25; IKA Co., Ltd., Staufen, Germany) at 10,000 rpm for 5 min and mixed using the Vortex Genie (SI-0236; Scientific Industries, Inc., New York, USA) for 5 min. Then, 2.5 mL chloroform and 2.5 mL pure water were added, with mixing for 2 min. After centrifugation (2410; Kubota Co., Ltd., Osaka, Japan) at 3000 rpm for 5 min. The sample was divided into three layers from top to bottom: water–methanol, microalgae, and lipid–chloroform. The lipid–chloroform layer was recovered and filtered through a 0.45-μm membrane. Most of the solvent was removed by rotary evaporation (40°C, vacuum condition), with the remaining solvent removed under flowing N2 gas. The obtained raw lipids were weighed and stored below 0°C for further analysis.

Analytical methodologies

Raw lipid content and relative extraction rate

To determine the two items, total water content and solid content of samples were measured firstly. Measurement of total water content was carried out by gravimetry after drying at a temperature of 105°C to a constant weight, and then cooling in desiccator to room temperature for weight measurement [5]. Then total water content and solid content were calculated by the following equations:

$$Solid\ content\ (\%) = \frac{W_{after}}{W_{before}} \times\ 100\% \tag{3}$$

$$Total\ water\ content\ (\%) = (1 -\ Solid\ content)\ \times\ 100\% \tag{4}$$

Where W_{before} and W_{after} are weight of samples before and after the drying respectively.

The raw lipid content represented raw lipid extracted per gram of microalgae (dry base), which was calculated by the following equation:

$$Raw\ lipid\ content\ (mg/g) = \frac{Mass\ of\ extracted\ raw\ lipid\ (mg)}{Solid\ content\ (\%)\ \times\ Mass\ of\ sample\ (g,\ wet\ base)} \tag{5}$$

The relative extraction rate of DME method in Eq. (6) represented the proportion of extracted raw lipid in total raw lipid (approximation) which was estimated by B&D method in the Section 5.

$$Relative\ extraction\ rate\ (\%) = \frac{Raw\ lipid\ content\ by\ DME\ method\ (mg/g)}{Raw\ lipid\ content\ by\ B\&D\ method\ (mg/g)} \times\ 100\% \tag{6}$$

Fatty acid methyl ester (FAME) content and composition

Because the extracted raw lipids contain not only lipid fractions (triglyceride and fatty acids) but also non-lipid fractions (wax esters, protein, steroids, steryl esters, hydrocarbons, terpenoids, polyhydroxyalkanoates, polycyclic aromatic hydrocarbons, and linear alkyl benzenes). And only the lipid fractions represent the saponifiable part of lipids are suitable for biodiesel, this part hence needs to be further analyzed [6]. The lipid fractions of the extracted raw lipids were converted into FAMEs by transesterification; the concentration was determined by gas chromatography–mass spectrometry (QP2010 Plus; Shimadzu Corp., Kyoto, Japan) equipped with DB column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Specifically, the raw lipids were redissolved in 5 mL dichloromethane (Guaranteed Reagent; Wako), subjected to methylation by mixing with 2 mL 14% BF_3–methanol solution (First Grade; Wako) at 65 °C for 40 min, and cooling to room temperature. The dichloromethane layer containing FAMEs was separated by adding 4 mL saturated aqueous NaCl solution (Guaranteed Reagent; Wako), and the dichloromethane was evaporated under flowing N2 gas. The recovered FAMEs were once again dissolved in dichloromethane for gas chromatography–mass spectrometry analysis; a 37-component FAME sample (Sigma–Aldrich Japan, Co., Ltd., Tokyo, Japan) was used as the standard (as shown in the Fig. 2). 10 μL sample extract was injected into a split/splitless injector operated in splitless mode at 250°C. The separation was performed using helium gas at a constant flow rate of 50 mL/min. The oven temperature was programmed as follows: held the initial temperature at 50°C for 1 min, then raised to 140°C at 20°C/min, then raised to 230°C at 10°C/min and finally ramped at 30°C/min to 280°C, and then held for 2 min. After the mass of FAME in each sample was measured by the GC/MS, its content was calculated by the following equation:

$$FAME\ content\ (mg/g) = \frac{Mass\ of\ FAME\ (mg)}{Solid\ content\ (\%)\ \times\ Mass\ of\ sample\ (g,\ wet\ base)} \tag{7}$$

Free and bound water contents

DSC (DSC-60; Shimadzu) was used to measure the free and bound water contents [7]. Samples (~10 mg) before and after DME treatment were cooled to −30°C in the instrument using liquid N_2.

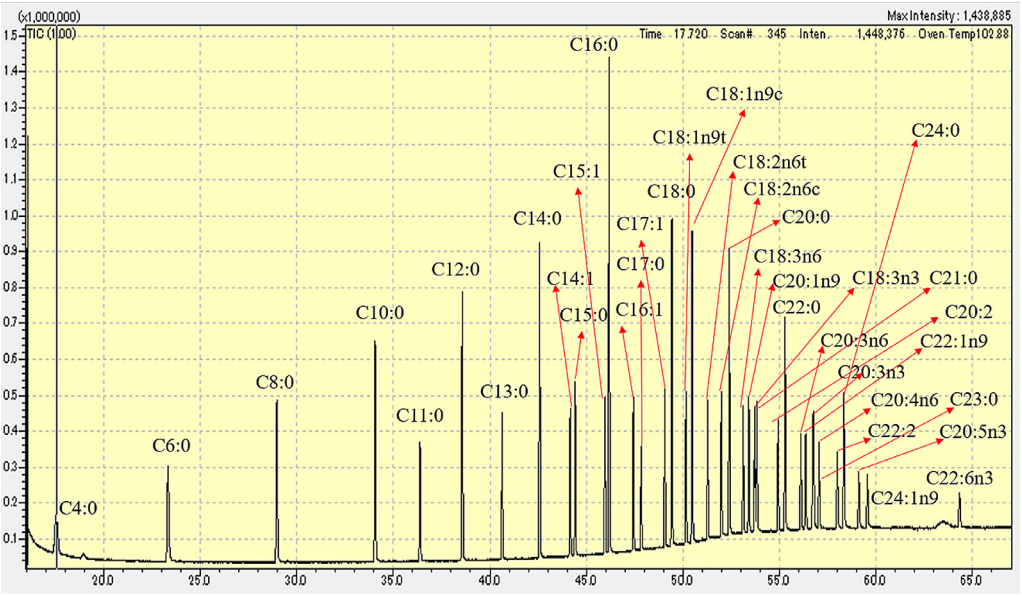

Fig. 2. Characteristic chromatogram of the 37-component FAME standard sample.

Assuming that all of the free water was frozen under this condition, the sample was then warmed to 20°C at 2°C/min. The endothermic transition near 0°C corresponded to the heat required to melt frozen free water; this enthalpy was used to determine the amount of free water present. The bound water was calculated by subtracting the free water content from the total water content.

Biomass samples, such as sludge, dewatered microalgae, contains both free water and bound water, which can be distinguished by DSC. During the measurement, sample is cooled to −30°C, then free water in sample will be frozen but bound water not, and by heating to above 0°C the frozen free water will thaw and absorb heat. DSC can record this melting Calorie to calculate the free water content, while bound water content can be obtained by subtracting free water from total water. The Fig. 3 shows the DSC measurement results of pure water. The pure water can be considered as whole free water. And the melting enthalpy of it is determined as – 339.34 J/g by our DSC.

Microalgal morphology

A digital microscope (VHX-900; Keyence, Osaka, Japan) was used to evaluate microalgal floc morphology before and after cell disruption. A scanning electron microscope (JED-2300 M; JEOL, Tokyo, Japan) operating at an accelerating voltage of 3.0/5.0 kV was used to observe the morphology of these cell-disruption treated microalgal cells before and after DME-extraction. Samples were freeze-dried (FDU-1100; Eyela, Tokyo, Japan) before SEM analysis, and the Auto Fine Coater (JFC-1600; JELO, Tokyo, Japan) was used to carbon-coat the samples.

Combustion characteristics of residues

TG-DTA of the microalgal residues after DME-extraction was conducted using the ThermoPlus TG8110 (Rigaku, Tokyo, Japan) from 20°C to 1000°C at 10°C/min under 50 mL/min flowing air. The elemental analyses of the C, hydrogen (H), N, and sulfur (S) contents of the residues were performed using an elemental analyzer (Micro Corder JM10; J-Science Labo Co., Ltd., Kyoto, Japan); the results were used to calculate the heating value [8].

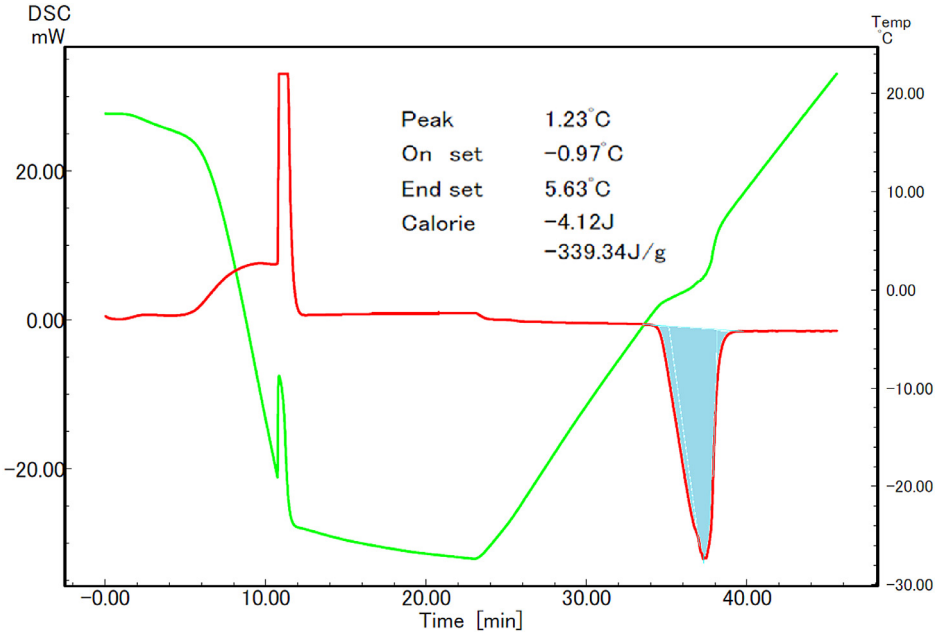

Fig. 3. Differential scanning calorimetry thermogram for pure water used to calculate the free and bound water contents.

Other analyses

Microalgal floc size was measured using a laser particle size analyzer (SALD-2200; Shimadzu). The properties of the microalgal filtrates with or without cell disruption were analyzed by 3D-EEM (RF-5300 pc; Shimadzu).

Statistical analysis

All data are presented as the mean ± standard deviation of three replicates including the lipid extraction tests in 4 and 5. The samples in 2 (a) and (b) respectively came from two different cultivation batches while the biomass concentration monitoring ensured the properties of the two batches were close. Data were subjected to analysis of variance using Microsoft Office Excel 2010 software, and the differences between means were assessed by Fischer's least significant difference test. Statistical significance was set at $p < 0.05$.

Conclusions

In this paper, a method is presented for evaluating the lipid extraction performance by subcritical DME under various cell disruptions. A practical example is given for evaluating the effect of microwave irradiation, water bath heating, acidification, homogenization, and ultrasonication on microalgae cell. For this purpose, a freshwater microalgal species T. obliquus was chosen, and several analyses were conducted on the extracted lipid, microalgal residuals and filtrates. The full-factorial design firstly gave the optimal conditions of DME dosage and extraction time, and was used as a comparison for sample with different water content. Based on these data, the samples achieved by different harvesting or cell disruptions having different water content can be compared. Lipid extraction by the common B&D method gave a standard for the total lipid content of microalgae, which can be used to calculate lipid extraction yield of DME method, and as a benchmark for comparison with other literature. As for the analytical methodologies, conventional indicators (raw lipid content and FAMEs content)

directly indicated the extraction performance, proposed free/bound water measurement characterized dewatering behavior by DME, thermal loss with heating value can show the potential of residuals as biosolid fuel, SEM/PSD/3D-EEM were proved to successfully display the disruption of cells. These results are important as, they can be a guide for other researches because the presented methods explained in detail how to prepare microalgal samples, and how to qualitatively/quantitatively analyze the cell disruption from multiple aspects. The proposed approach is just one application of T. obliquus with five common cell disruptions, and it can be applied in other microalgae species and other cell disruption treatments.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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