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Short Communication

Direct transesterification of fresh microalgal cells

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HIGHLIGHTS

- A scale of 300 μg microalgae transesterification.
- Direct transesterification of fresh microalgal cells.
- Application of fresh cells transesterification method to 8 microalgal species.

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ABSTRACT

Transesterification of lipids is a vital step during the processes of both biodiesel production and fatty acid analysis. By comparing the yields and fatty acid profiles obtained from microalgal oil and dry microalgal cells, the reliability of method for the transesterification of micro-scale samples was tested. The minimum amount of microalgal cells needed for accurate analysis was found to be approximately 300 μg dry cells. This direct transesterification method of fresh cells was applied to eight microalgal species, and the results indicate that the efficiency of the developed method is identical to that of conventional method, except for *Spirulina* whose lipid content is very low, which means the total lipid content should been considered.

1. Introduction

Transesterification represents a key approach in the fatty acid analysis and quantification in microalgal research (Griffiths et al., 2010). The conventional method for fatty acid analysis begins with lipid extraction using organic solvents followed by transesterification of extract (Kasim et al., 2010). Inevitably, the lipid content obtained using different lipid extraction techniques varies dramatically (Lohman et al., 2013), and therefore, the products of transesterification of different extracts also differ. To circumvent this problem, a method for direct transesterification of whole cells without extraction was developed (Wahlen et al., 2011). However, several milligrams or even larger amounts of lyophilized microalgae are usually demanded for such transesterification reactions. A transesterification approach that uses only micro-scale samples and can be applied to fresh cells would be valuable for daily fatty acid analysis in microalgal cultivation or fatty acid analysis in strain screening and physiological studies of species. Bigelow et al. (2011) had developed a micro-scale sample protocol using boron trifluoride as transesterification catalysis for lipid analysis. Previous studies have demonstrated that transesterification of wet biomass in biodiesel processing saves the energy consumed in biomass drying and lipid extraction (Ehimen et al., 2010). Griffiths et al. (2010) achieved the direct transesterification of wet biomass via the addition of a water scavenger to ensure anhydrous reaction conditions. Wet biomass transesterification under supercritical conditions also has been explored (Reddy et al., 2014). However, to date, a convenient and reliable transesterification method for laboratory use in fatty acid analysis is still a technological requirement. In this study, we investigated a direct transesterification approach using micro-scale samples of microalgal biomass and fresh microalgal cells without dehydration. The effects of temperature, catalyst concentration, and water content on the transesterification efficiency were investigated using eight species of microalgae.

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2. Methods

2.1. Materials

Eight species of microalgae were used in this study. Details can be found in Supplementary Material. Chromatographic grade *n*-hexane and methanol were purchased from J&K Scientific Ltd. (China). Chloroform and sulfuric acid (purity, 95–98%) was analytical grade and obtained from Tianjin Kemiou Chemical Reagent Co., Ltd. (China). Methyl heptadecanoate (C17-ME) and glyceryl trioleate (C18-TAG) were purchased from Sigma–Aldrich (USA).

2.2. Preparation and treatment of microalgae

Microalgal cells were harvested by centrifugation at 4000 rpm for 10 min once reaching a stationary phase, except for *Spirulina,* which were harvested by two centrifugation steps. After centrifugation, the fresh microalgal cells, which are referred to as microalgal paste, were obtained by discarding the supernatant. Microalgal pastes from marine species and fresh water species were washed twice with 0.5 mol L^{-1} NH_4HCO_3 or distilled water respectively, and dried at 60 °C until the weight of samples remained constant (Yao et al., 2012). Samples were then ground into a powder.

2.3. Lipid extraction

Total lipids from 100 mg microalgae were extracted using 2 mL chloroform/methanol (v/v: 2/1) (Bligh and Dyer, 1959), ultrasonic treatment for 10 min and centrifugation at 4000 rpm for 5 min. The supernatants were then collected into pre-weighted centrifuge tubes. This process was repeated three times. The collected supernatants were dried under nitrogen flow and then at 60 °C until the weight of samples remained constant.

2.4. Transesterification

The samples were weighed and moved to 10 mL flasks. Then, 5 mL H_2SO_4–methanol (v/v H_2SO_4/methanol) was added, and the flask was stirred at a specific temperature for a specific amount of time with refluxing. After the specific time period, the flask was cooled to room temperature. Next, 2 mL of hexane and 0.75 mL of distilled water were added to the flask and mixed for 30 s on a vortex mixer. The mixture formed two phases, and the upper hexane layer contained the fatty acid methyl esters (FAMEs). The hexane layer was transferred to a new vial and mixed with the internal standard C17-ME for analysis by gas chromatography (GC).

Micro-scale samples for transesterification were prepared following a different procedure from that described above. After adding hexane and distilled water and vortex mixing the solution for 30 s, precisely 1.5 mL of the upper layer was transferred to a 2 mL vial and dried completely under a nitrogen flow. The FAMEs were re-dissolved in 120 μl of hexane for GC analysis.

2.5. Composition analysis and yield of FAMEs

FAME analyses were carried out by an Agilent 6890 GC instrument was equipped with a flame-ionization detector (FID) and a DB-23 capillary column (Agilent Technologies, USA, 30 m × 0.32 mm × 0.25 μm) (Wang et al., 2014). FAME yield was calculated using the following equation:

$$\text{FAME Yield}(\%) = (\text{FAME mass})/(\text{oil mass}) \times 100\% \quad (1)$$

All the measurements of the values used in the tables and figures represent the average ± SD of four individual replicates during the whole experiment.

3. Results and discussion

3.1. Optimal conditions of transesterification of triacylglycerides (TAG)

The effects of temperature, reaction time, and concentration of H_2SO_4 on the transesterification efficiency were studied using a substrate of C18-TAG standard. The optimal transesterification conditions were determined to be a temperature of 70 °C, a catalyst concentration of 2% H_2SO_4 in methanol, and a reaction time of 1 h. In consideration of the effect of the volume of methanol on the conversion of TAG, the volume of methanol was in large excess and thus did not have effect on the transesterification in the present study. These conditions were applied in subsequent experiments. Details can be found in Supplementary Material.

3.2. Transesterification of micro-scale samples of dry microalgal cells

Transesterification of extracted microalgal oil, 5 mg samples of dry cells, and micro-scale samples of dry cells was carried out. According to our experience in the analysis of microalgal fatty acids, the ±10% variation in the percentage of each fatty acid is acceptable. The oil samples, 5 mg samples of dry cells, and micro-scale samples of dry cells were prepared from *Isochrysis zhangjiangensis* biomass. The results obtained for the oil samples, 5 mg samples, and micro-scale samples of 300 μg are shown in Table 1. Relative fatty acid percentages within ±10% variability were obtained from all three microalgal forms. However, 300 μg of dry *I. zhangjiangensis* cells was found to be the minimum quantity required for this approach. When the amount of dry cells was less 300 μg, the relative fatty acid percentages varied dramatically, and the analysis results differed greatly from the results obtained with samples containing more than 300 μg of dry cells. Thus, a sample of less than 300 μg led to non-negligible whole process error, including error related to the fatty acid content of the specific microalgal species.

Table 1
Fatty acid composition (%) obtained by transesterification of microalgal oil, 5 mg samples of dry cells, micro-scale samples of dry cells and paste of *Isochrysis zhangjiangensis*.

Fatty acid	Extracted lipid	5 mg dry cells	300 μg dry cells	Fresh cells (paste)	±10% variation range[a]
C14:0	16.4 ± 0.1	16.2 ± 0.1	15.0 ± 0.4	16.0 ± 0.3	14.6–17.8
C16:0	10.4 ± 0.2	11.0 ± 0.2	11.3 ± 0.3	11.2 ± 0.3	9.9–12.1
C16:1n7	8.3 ± 0.1	8.3 ± 0.2	8.1 ± 0.1	8.1 ± 0.1	7.5–9.1
C18:1n9	10.1 ± 0.2	9.6 ± 0.1	8.9 ± 0.1	10.0 ± 0.1	8.6–10.6
C18:2n6	9.7 ± 0	9.6 ± 0.2	9.6 ± 0.2	9.6 ± 0.1	8.6–10.6
C18:3n3	15.4 ± 0.1	15.5 ± 0.4	15.2 ± 0.3	15.3 ± 0.2	13.9–17.1
C18:4n3	15.3 ± 0.2	14.8 ± 0.1	15.7 ± 0.3	14.8 ± 0.1	13.3–16.3
C18:5n3	2.8 ± 0.1	3.1 ± 1.1	3.4 ± 0.4	2.3 ± 0.1	2.8–3.4
C20:5n3	2.9 ± 0	2.8 ± 0.3	2.6 ± 0.3	3.2 ± 0.4	2.5–3.1
C22:6n3	8.8 ± 0.1	9.2 ± 0.3	10.1 ± 0.3	9.5 ± 0.2	8.3–10.1
Total	100	100	100	100	100

[a] The variation in fatty acid percentage was based on the 5 mg dry cell sample.