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# Optimization of the transesterification reaction of microalgal *Monoraphidium* sp

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ABSTRACT

This study optimized biodiesel production by *Monoraphidium* sp. in terms of fatty acid methyl ester (FAME) yield by varying four variables (volume of methanol, reaction temperature, reaction time, and ultrasound power) using a Box-Behnken design (BBD). Within the experimental range, the volume of methanol was found to be the most important factor, having a positive influence on the FAME yield. The test variables affected the FAME yield, and the optimal condition varied between alkali- and acid-catalyzed transesterification. Both types of transesterification reaction occurred at their optimal catalyst concentrations, 0.5% NaOH and 2% $H_2SO_4$, respectively, for both dry and wet microalgal biomass. The FAME yield with the acid-catalyzed reaction was better than that with the alkali-catalyzed reaction. The combined alkali-and-acid-catalyzed transesterification reactions enhanced the FAME yield. The microalgae produced fatty acids, comprising mainly palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), with palmitic acid (C16:0) and linoleic acid (C18:2) being the most abundant.



## 1. Introduction

Biodiesel (fatty acid methyl esters, FAMEs), a clean-burning fuel, is produced via esterification and transesterification of free fatty acids (FFAs) and triglycerides. It is typically made from renewable biological resources such as grease, vegetable oils, or animal fats. Microalgae are another source of triglycerides, which can be converted into biodiesel [1]. Microalgae served as non-food feedstocks for making biodiesel due to its higher lipid content and faster growth than those of energy crops. On an area basis, microalgae can produce 15–300 times more oil for biodiesel production than traditional oilseed crops. Microalgae can contribute to not only a reduction in land requirements but also lower environmental impacts. Microalgal oils suitable for making biodiesel are common [2].

Four major ways have been adopted to produce biodiesel, including direct use and blending of raw oils, micro-emulsions, thermal cracking, and transesterification. The most important step in the overall process of biodiesel production is the transesterification reaction, which related to both energy- and cost-intensive. Transesterification can be catalyzed by both alkali and acid catalysts. NaOH, $CH_3ONa$, $CH_3OK$, and KOH are the most commonly used alkali catalysts [3]. For acid-catalyzed systems, sulfuric acid, HCl, $BF_3$, $H_3PO_4$, and organic sulfonic acids have been used [4]. The alkali-catalyzed reaction is about 4000 times faster than the acid-catalyzed reaction. However, the performance of alkali catalysts is strongly affected by the presence of FFAs in the feedstock.

There are several key factors affecting the FAME yield obtained from transesterification reaction, including alcohol quantity, reaction temperature, reaction time, and catalyst concentration. Although the theoretically alcohol to triglyceride molar ratio is 3:1, an excess of alcohol is generally used for making biodiesel to ensure that the oils or lipids are completely converted to esters. However, some studies reported that further increasing the alcohol amount beyond the optimal ratio does not increase the FAME yield, but it increases the cost of alcohol recovery [5,6]. D'Oca et al. [7] studied the extraction of lipids from microalgae *Chlorella pyrenoidosa* using Soxhlet extraction, magnetic stirring, and an ultrasonic bath with five solvents: a mixture of chloroform/methanol (2:1 v/v), methanol, chloroform, ethanol, and hexane. They found that chloroform/methanol (2:1 v/v) led to the highest lipid extraction, followed by methanol, chloroform, ethanol, and hexane. Sheng et al. [8] demonstrated that a mix of chloroform/methanol had the highest lipid recoveries for cyanobacterium *Synechocystis*. Additionally, the

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catalyst concentration for FAME yield has been investigated. The catalyst concentration increased the conversion of triglycerides, increasing the FAME yield. However, it might have an optimal catalyst concentration for the triglycerides transesterificated into FAMEs among various microalgal spices. The FAME yield obtained from the diatom C. *gracilis* cells was highest when the catalyst ($H_2SO_4$) concentration is 2.0% (v/v), decreasing slightly with a further increase in the catalyst concentration [5]. Patil et al. [9] reported that around 2.0 wt% of catalyst (KOH/MeOH) resulted in the maximum FAME yield of *Nannochloropsis* sp under microwave-assisted transesterification for dry algal biomass.

The FAME yield also significantly depends on reaction time and temperature. Leung et al. [6] reported that the highest FAME yield of both neat Canola oil and used frying oil was found at a reaction time of 15 min at 70 °C, and then remains relatively constant with a further increase in the reaction time. The FAME yield decreased because excess reaction time led to backward transesterification reaction, as a result, accelerated the saponification reaction of triglycerides and caused the extraction solvent to leak out via vaporization [10].

The relationship between the factors of transesterification might be complicated and difficult to identify from simple experiments. The aim of the present study was first to determine the optimal alkali and acid catalyst concentrations, and then to examine the correlations among the factors (methanol amount, reaction temperature, reaction time, and ultrasound power) that affect the FAME yield using the Box-Behnken design (BBD) method to optimize biodiesel production. A comparison of the effects of alkali- and acid-catalyzed transesterification reactions on the FAME yield of wet microalgal biomass was also conducted.

## 2. Materials and methods

### 2.1. Sampling

Water samples (10 L) were collected form the secondary effluent of wastewater treatment plant of Neipu industrial park in southern Taiwan (22°31′43″N; 120°37′46″E). The wastewater treatment plant treat mainly food processing wastewater using activated sludge process with capacity of 1400 $m^3$, covering overall area of 2.8 ha. The composition of the effluent was in average of 8.2 pH, 2.9 mg/L of suspended solid, and 16.1 mg/L of chemical oxygen demand. A 1000-mL of each sample, totally 3 L composite water sample was collected. The water sample was stored in a transparent plastic bottle in a dark environment and sent to the laboratory within 24 h for algal cell identification and isolation. The strains were identified for taxonomy using Standard Methods [11] and morphology.

### 2.2. Microalgal culture

Microalgal species, *Monoraphidium* sp., which has high lipid content (40–50%), was isolated from the water sample and cultured in BG-11 medium. The stock cultures were maintained routinely on BG-11 medium with 1.5% agar by regular subculturing for 30 d. The microalgae were subjected to purification by serial dilution followed by plating. The microalgae from single colonies were then propagated in flasks containing BG-11 medium on a shaker (250 rpm) at 25 °C with 5 klux of illumination in incubator. The microalgae that reached enough seed culture was inoculated in batch mode in a 1-L modified serum bottle containing 600 mL of BG-11 medium. Then, axenic cultures of *Monoraphidium* sp. were grown in batch mode in a 5-L modified serum bottle containing 4 L of sterilized algal medium. The bottles were placed in an incubator that was maintained at 25 °C and continuously provided with 5 klux of illumination. $CO_2$ (3% vol.) was continuously supplied to the cultures every day. Cultures were harvested in the log growth phase after 7 days for experiments.

### 2.3. Extraction of total lipid and preparation of FAMEs

A stock culture of *Monoraphidium* sp. cells was collected by centrifugation at 9000*g* for 15 min (CR22G III, Hitachi, Japan). The precipitated microalgal cells were then washed and resuspended in deionized water in triplicate. The wet microalgal biomass was collected by centrifugation again and properly stored for testing. The remaining centrifuged microalgal biomass was then dried in a freeze dryer at −80 °C at about 30 Pa. The microalgal total lipids were extracted with *n*-hexane/methanol (2/1, v/v) in a Soxhlet extractor (S-416, Gerhardt, Germany) and quantified gravimetrically. The lipid content (g/g) is expressed as the dry weight of the microalgal biomass.

The rest of Freeze-dried biomass (0.102 ± 0.0002 g) was placed in 50-mL Teflon-capped Pyrex tubes for the transesterification reaction. The biomass samples were firstly reacted with a premixed homogeneous solution of alkali or acid catalyst and methanol at 60 °C for 3 h. Both the methanol volume and the concentration of sulfuric acid (acid-catalyzed) or sodium hydroxide (alkali-catalyzed) were varied to determine the optimal amount on FAME yield. After the optimal alkali and acid catalyst concentrations had been determined, depending on the experimental design, 3.0–21 mL of alkali or 8.0–20 mL of acid catalyst (NaOH:methanol

**Table 1**
Box-Behnken experimental range and levels.

| Run | Variable | | | | FAME yield | |
|---|---|---|---|---|---|---|
| | A | B | C | D | Alkali-catalyzed | Acid-catalyzed |
| 1 | −1 | −1 | 0 | 0 | 13.61 | 30.02 |
| 2 | 1 | −1 | 0 | 0 | 80.47 | 47.04 |
| 3 | −1 | 1 | 0 | 0 | 14.11 | 29.09 |
| 4 | 1 | 1 | 0 | 0 | 76.93 | 64.35 |
| 5 | 0 | 0 | −1 | −1 | 51.63 | 39.17 |
| 6 | 0 | 0 | 1 | −1 | 57.04 | 60.90 |
| 7 | 0 | 0 | −1 | 1 | 57.35 | 44.52 |
| 8 | 0 | 0 | 1 | 1 | 61.79 | 49.21 |
| 9 | 0 | 0 | 0 | 0 | 46.80 | 63.37 |
| 10 | −1 | 0 | 0 | −1 | 14.45 | 51.98 |
| 11 | 1 | 0 | 0 | −1 | 75.28 | 58.08 |
| 12 | −1 | 0 | 0 | 1 | 15.50 | 50.43 |
| 13 | 1 | 0 | 0 | 1 | 73.82 | 66.26 |
| 14 | 0 | −1 | −1 | 0 | 48.07 | 32.42 |
| 15 | 0 | 1 | −1 | 0 | 46.00 | 68.98 |
| 16 | 0 | −1 | 1 | 0 | 61.19 | 52.67 |
| 17 | 0 | 1 | 1 | 0 | 55.26 | 59.34 |
| 18 | 0 | 0 | 0 | 0 | 48.48 | 63.01 |
| 19 | −1 | 0 | −1 | 0 | 19.38 | 34.43 |
| 20 | 1 | 0 | −1 | 0 | 85.78 | 69.28 |
| 21 | −1 | 0 | 1 | 0 | 18.02 | 60.27 |
| 22 | 1 | 0 | 1 | 0 | 92.53 | 72.83 |
| 23 | 0 | −1 | 0 | −1 | 62.59 | 27.22 |
| 24 | 0 | 1 | 0 | −1 | 58.17 | 61.54 |
| 25 | 0 | −1 | 0 | 1 | 62.99 | 43.71 |
| 26 | 0 | 1 | 0 | 1 | 57.34 | 51.20 |
| 27 | 0 | 0 | 0 | 0 | 46.12 | 69.00 |

| Variable | Symbol | Code levels | | | | | |
|---|---|---|---|---|---|---|---|
| | | alkali catalyst | | | acid catalyst | | |
| | | −1 | 0 | 1 | −1 | 0 | 1 |
| 0.5% NaOH/MeOH, 2% $H_2SO_4$/MeOH (mL) | A | 3 | 12 | 21 | 8 | 14 | 20 |
| Reaction temp. (°C) | B | 20 | 40 | 60 | 40 | 60 | 80 |
| Reaction time (min) | C | 5 | 10 | 15 | 15 | 20 | 25 |
| Ultrasound power (W) | D | 54 | 108 | 180 | 54 | 108 | 180 |

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