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Short Communication

Enhancing the various solvent extraction method via microwave irradiation for extraction of lipids from marine microalgae in biodiesel production

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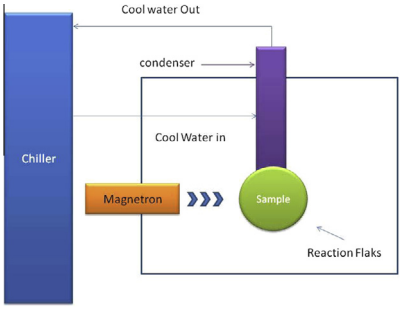
HIGHLIGHTS

- Solvent extraction methods Hara and Radin, Folch, Chen and Bligh and Dryer are used.
- The methods were modified by microwave irradiation for extraction of lipids.
- Microwave irradiation enhanced the lipid extraction for production of biodiesel.
- Microwave irradiated Hara and Radin show the highest lipid yield for *Tetraselmis* sp.
- *Nannochloropsis* sp. is best extracted using microwave irradiated Folch method.

GRAPHICAL ABSTRACT



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ABSTRACT

The types of microalgae strains and the method used in lipid extraction have become crucial factors which influence the productivity of crude oil. In this paper, *Nannochloropsis* sp. and *Tetraselmis* sp. were chosen as the strains and four different methods were used to extract the lipids: Hara and Radin, Folch, Chen and Bligh and Dyer. These methods were performed by using conventional heating and microwave irradiation methods. Results revealed that highest lipid yield from the different species was obtained using different extraction methods; both under microwave irradiation. The lipid yield for *Tetraselmis* sp. and *Nannochloropsis* sp. was highest when Hara and Radin (8.19%), and Folch (8.47%) methods were used respectively under microwave irradiation. The lipids extracted were then transesterified to biodiesel and the quality of the biodiesel was analyzed using the gas chromatography.

1. Introduction

The demand for energy in developing countries has increased tremendously due to industrialization, fast growing population and rapid modernization. Currently, the energy demand is mostly met from non renewable resources for example natural gas, petrochemicals and coal (Adholeya and Dadhich, 2008). Such high dependency on fossil fuels results in rapid depletion in the oil reserves. Thus, it is necessary to find measures to supply sustainable and renewable fuels to alleviate these problems and enhance economic prosperity and sustainability. Micro-biodiesel was being considered as one of the best sustainable sources to replace petroleum-based fuels (Luque et al., 2010 and Chisti, 2008).

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There are four methods of solvent extraction that are commonly used which are Radin and Hara, Bligh and Dyer, Folch and Chen. In these four methods, different solvents are used for lipid extraction. The Bligh and Dyer (1959) method is 3 step solvent extraction and well established. It is a simple standard method where the total lipids can be determined easily and samples can be analyzed directly with no pre-drying necessary and lipids can be used for further determinations. However the disadvantages are adverse effects of chloroform on the environment (EU regulation controlling chlorinated solvents) and laborious (filtration, etc.). The Folch et al. (1957) method is a one step solvent extraction and is commonly used. It allows the determination of total lipids but has similar disadvantages like the Bligh & Dyer method. Like the Folch method, the Hara and Radin (1978) is a 1-step solvent extraction. This method uses solvents which are less toxic in solvents and cheaper than chloroform and methanol, no interference in processing by proteolipid protein, extract contains less non-lipids compared to chloroform–methanol extracts of Folch. However this method is laborious and there is no extraction of gangliosides (a minor fraction of total lipids). Chen et al. (1981) modified the Folch et al. method using dichloromethane instead of chloroform under room temperature so as to avoid the use of the toxic substance (chloroform).

Microwave heating (MW) is a non-contact heat source, which heats the overall target reactants simultaneously as compared to conductive heating. It has recently been used for extraction of lipids from marine microalgae using common solvents (Lee et al., 2010). In conventional heating; heat transfer occurs from the outside to the inside whilst mass transfer occurs from the inside to the outside. In microwave assisted solvent extraction, the mass and heat transports occur from the inside of the extracted material to the bulk solvent (Virot et al., 2008). In addition, Lee et al. (2010), Cheng et al. (2013) and Javed (2012) have reported on the microwave assisted extraction from microalgae but they focused mainly on the solvent extraction using the Bligh and Dyer method only. In the work of Lee et al. (2010), the lipids of *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. were extracted using a mixture of chloroform and methanol in the ratio of 1:1 using autoclaving, bead-beating, microwaves, sonication, and a 10% NaCl solution. Cheng et al. (2013) extracted lipids from *Chlorella* PY-ZU1 using the improved Bligh–Dyer method using a chloroform:methanol (1:1, v/v) mixture while Javed (2012) extracted extracted lipids from *Nannochloropsis* sp. using the Soxhlet extraction method. Most of the other methods have been used mainly in the extraction of oils from plants.

In this research, the lipid was extracted from the marine microalgae using 4 methods: (i) Hara and Radin, (ii) Folch et al., (iii) Chen et al. and (iv) Bligh and Dryer. These four methods were then modified by subjecting the heating process to microwave irradiation. The microalgae used in this study were *Nannochloropsis* sp. and *Tetraselmis* sp. (Teo et al., 2014) due to their high lipid content.

2. Methods

2.1. Marine microalgae cultures

The strain of microalgae *Nannochloropsis* sp. and *Tetraselmis* sp. were originally obtained from the culture collection from Borneo Marine Research institute (BMRI), Universiti Malaysia Sabah, Malaysia.

2.2. Marine microalgae cultivation

The *Nannochloropsis* sp. and *Tetraselmis* sp. cells were cultured in sterilized seawater enriched with Walne's medium which contains: 100 g $NaNO_3$, 1.3 g $FeCl_3{\cdot}6H_2O$; 0.36 g $MnCl_2{\cdot}4H_2O$; 33.6 g H_3BO_3; 45 g $Na_2{\cdot}EDTA$; 20 g $NaH_2PO_4{\cdot}2H_2O$; 2.1 g $ZnCl_2$; 2 g $CoCl_2{\cdot}6H_2O$; 0.9 g $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$; 2 g $CuSO_4{\cdot}5H_2O$; 0.001 g Vitamin B_{12}; 0.001 g Vitamin B_1 and 0.2 mg Biotin per liter. *Nannochloropsis* sp. and *Tetraselmis* sp. were also cultured in 2 L flasks and the conditions were maintained at 23 ± 0.5 °C, pH 8 ± 0.2 under a light intensity of 100 μmol m^{-2} s^{-1} (white fluorescence lamp) with 24:00 light–dark cycle and aeration condition for 8 days. The light intensity was measured with a quantum sensor connected to Light Scout Dual solar quantum light meter. Each experiment was performed in duplicates so as to ensure reproducibility of results.

2.3. Harvesting

The 2 L marine microalgae were dewatered by centrifugation (Hermle labortechnik GmbH, 2323 K) at 8000 rpm for 15 min. The supernatant consisting of the culture medium was removed. As a result, the 100 ml concentration microalgae (from 2 L culture) were collected.

2.4. Lipid extraction

There are four methods of extraction used; Hara and Radin (Hara and Radin, 1978), Folch (Folch et al., 1957), Chen (Chen et al., 1981) and Bligh and Dyer (Bligh and Dyer, 1959). The protocol for these methods is summarized in Table 1. The four original methods were performed in conventional heating and repeated in microwave irradiation. The microwave irradiation was performed in MAS-II Microwave Synthesis Workstation. Temperature of reaction mixture was measured directly by using infrared (IR) thermocouple to maintain at 65 °C and microwave radiation at 500 watt. A volume of 15 ml concentrated wet marine microalgae was used in all the experiments. All experiments were carried out in duplicates for ensure the reproducibility results.

2.4.1. Hara and Radin method

The biomass (15 ml) was mixed and homogenized with 20 mL of isopropanol and placed under microwave irradiation for 5 min at 65 °C. Hexane (30 mL) was added and the mixture was again placed under microwave irradiation for another 5 min at 65 °C. The mixture was then placed on an orbital shaker to ensure complete reaction.

2.4.2. Folch et al. method

The biomass (15 ml) was mixed and homogenized with 25 ml of methanol. The mixture was then subjected to microwave irradiation for 5 min at 65 °C. Chloroform (50 ml) was added to the mixture and placed under microwave irradiation for another 5 min at 65 °C. The mixture was placed on an orbital shaker to ensure complete reaction.

2.4.3. Chen et al. method

The biomass (15 ml) was mixed and homogenized with 25 ml of methanol under microwave irradiation for 5 min at 65 °C. Dichloromethane (50 mL) was added and the mixture was placed under microwave irradiation for 5 min for another at 65 °C. The mixture was then placed on an orbital shaker to ensure complete reaction.

2.4.4. Bligh and Dyer method

The biomass (15 ml) was mixed and homogenized with 25 ml of methanol, 12.5 ml of chloroform and 5 ml of water. The subjected mixture was under microwave irradiation for 5 min at 65 °C. Chloroform (12.5 ml) and a solution of 1.5% w/v sodium sulfate (12.5 ml) were added to the mixture. The mixture was placed